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CYLINDROCARPON EHRENBERGI WR., AND OTHER SPECIES, AS ROOT PARASITES OF ALFALFA AND SWEET CLOVER IN ALBERTA[1]

BY M. W. CORMACK[2]

Abstract

Cylindrocarpon Ehrenbergi appears to be one of the most important pathogenic fungi associated with early spring injury of roots of alfalfa and sweet clover in Alberta. It occurs in virgin and cultivated soils. It produces distinctive symptoms, and is highly pathogenic in the early spring, but less virulent during the growing season. It can invade unwounded roots through lenticels or the basal tissues of branch roots, or by direct penetration. It is also pathogenic on roots of *Trifolium* spp. This species has not been previously reported on the legume forage crops, and very little is known concerning its parasitism on other plants.

On the roots of alfalfa and sweet clover *C. obtusisporum* is slightly to moderately pathogenic, *C. radicicola* is very weakly pathogenic, and *C. olidum* is non-pathogenic. These species occur infrequently on diseased roots, and usually in association with *C. Ehrenbergi*. *C. radicicola* has been reported as an important root parasite of other plants.

Isolates of *C. Ehrenbergi* differ in degree of pathogenicity, and there is some evidence of host specialization. They also differ markedly in morphological and cultural characteristics, which, however, do not appear to be correlated with their parasitic abilities. The temperature range for growth of *C. Ehrenbergi* in pure culture is from −2° to 32° C., but different isolates do not have the same optima. Isolates with an optimum at about 19° C. caused the most damage in the early spring, while one which grew best at 24° C. proved the most virulent during summer. The optimum hydrogen ion concentration for growth of *C. Ehrenbergi* varies with the medium employed. Growth and spore germination studies indicate that the iso-electric point for the fungus lies at approximately pH 5.1.

Most of the commonly grown varieties of alfalfa and sweet clover are susceptible to attack by *C. Ehrenbergi*, but resistant species like *Medicago falcata* may prove valuable as plant breeding material. Apparently cereal crops are not attacked by the pathogen, therefore they should be grown for several years in severely infested fields.

During recent years, root rot of alfalfa and sweet clover has become increasingly prevalent in Alberta. Most of the damage occurs at the end of the dormant period when the plants are particularly susceptible to attack by certain pathogenic fungi. Later in the season the same or other fungi may cause a less destructive root rot of the growing plants.

Previous studies on the fungi associated with these root rots have been concerned with *Plenodomus Meliloti* and *Sclerotinia* sp. (23, 24). These commonly occurring pathogens are especially injurious in the early spring, and,

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as shown by Sanford (23), *P. Meliloti* ceases to attack the roots when growth starts. Further isolation and pathogenicity studies have shown that other fungi are involved. *Cylindrocarpon Ehrenbergi* was chosen for detailed study because of its wide distribution and frequent occurrence on diseased roots. The less commonly isolated species *C. obtusisporum*, *C. radicicola* and *C. olidum* were included in the present investigation, which deals primarily with the taxonomy of the isolates, their pathology on roots of alfalfa and sweet clover, and certain aspects of their physiology.

Symptoms

C. Ehrenbergi usually produces characteristic symptoms on infected roots of alfalfa and sweet clover in the early spring. Periodic observations made on plants grown in artificially infested soil in the field have shown that infection begins at the first sign of thawing in the soil. The partially frozen soil is permeated with whitish mycelium, which rapidly invades the previously sound roots. This mycelium gradually becomes less evident and disappears when the soil becomes warm. Three representative stages of infection are shown in Fig. 1, A. The infected areas on the roots have first a water-soaked appearance, but soon increase in size, turn light brown in color, and finally dark brown. When infection is severe, the entire root system is often rotted within a week or two after the first sign of infection. Compact white masses of mycelium later form in the ruptured cork covering of the root, and develop into the characteristic sclerotia-like stromata of the fungus (Fig. 1, B and C; Plate I, D). These bodies are not uniform in color or consistency, but are usually salmon-orange in color and fairly hard and brittle.

The degree of infection with *C. Ehrenbergi* varies greatly from year to year, but sweet clover usually suffers more damage than alfalfa. By late May, severely attacked plants are dead and have roots which are either partially rotted (Fig. 1, B), or have been converted into decayed, misshapen masses (Fig. 1, C). The rotted tissues are more or less completely covered with an irregular layer of the aggregated stromata. At the same time, check plants, growing in adjacent plots of non-infested soil, are starting vigorous growth, and have sound roots (Fig. 1, B). When light infection occurs, irregular brown lesions of varying size are formed on the main tap or lateral roots. Stromata are usually aggregated near the centre of these lesions, or may be buried in the decayed tissues at the margin.

Crown rot is another type of injury frequently caused by *C. Ehrenbergi* in the early spring. Rotted areas of varying size appear at the crown, and involve all or part of the crown buds (Fig. 1, D). The plant dies when the crown buds are destroyed, but the root system may remain undecayed for some time.

During the growing season, natural infection of alfalfa and sweet clover roots by *C. Ehrenbergi* has seldom been observed. Likewise, the infection resulting from artificial inoculation of the roots is much less severe than that occurring in the early spring. The small, cinnamon-brown lesions produced

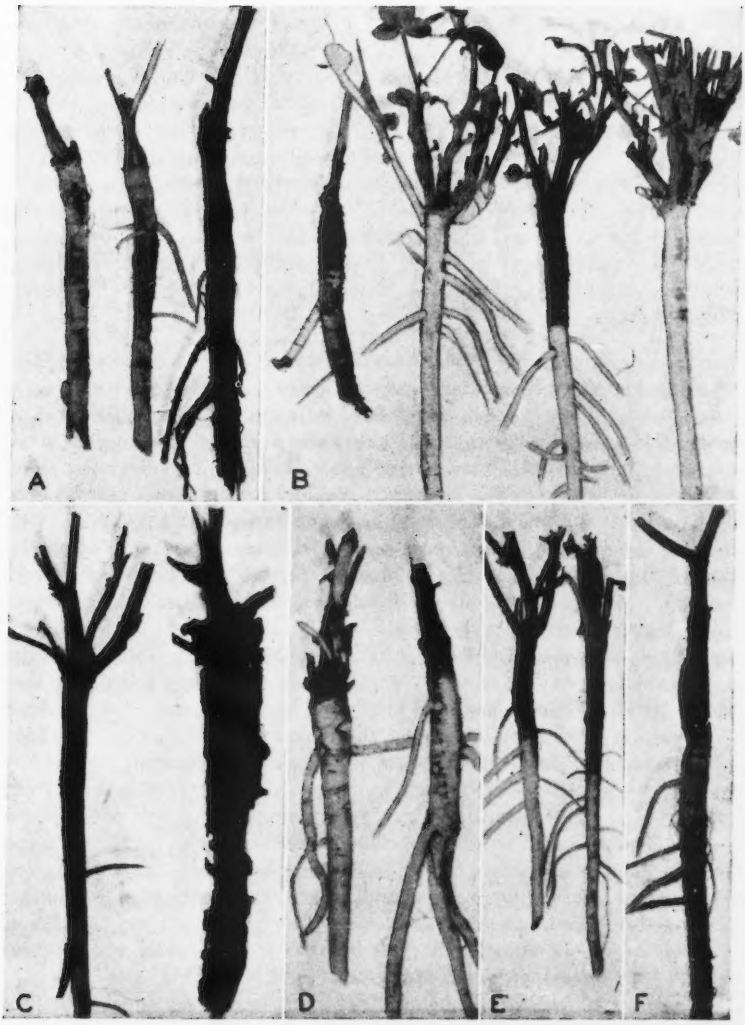

FIG. 1. A–D. *Roots attacked in the early spring by Cylindrocarpon Ehrenbergi.* A. *Three stages of infection of sweet clover roots.* B. *Typical symptoms. Left, Arctic sweet clover, diseased and healthy; right, Grimm alfalfa, diseased and healthy.* C. *Severely attacked roots. Left, Grimm alfalfa; right, Arctic sweet clover.* D. *Crown infection of sweet clover.* E. *Early spring infection of alfalfa roots by C. Ehrenbergi (left), and by C. obtusisporum (right).* F. *Sweet clover root infected near the crown by C. Ehrenbergi and at the bottom by Sclerotinia sp.*

on the roots of growing plants are slightly sunken toward the centre and have a narrow dark border. Stromata usually appear on the lesions within 30 days after inoculation.

C. obtusisporum seldom attacks inoculated roots as severely as does *C. Ehrenbergi*, and it does not produce stromata, but otherwise the symptoms are indistinguishable (Fig. 1, E). When *C. Ehrenbergi* does not form stromata on infected roots, the damage may be confused with that caused by other fungi in the early spring. There is usually no difficulty, however, in recognizing the pycnidia of *Plenodomus Meliloti* (23), or the pulpy rot and sclerotia produced by *Sclerotinia* sp. (8). Individual roots are sometimes attacked by more than one pathogen (Fig. 1, F). When mixed infection occurs, or distinctive symptoms are not produced, isolations are necessary to determine the fungi involved.

Isolation Studies

When infected roots are plated on agar, *Cylindrocarpon* spp. usually grow out very slowly, and are easily suppressed or overgrown by other fungi or bacteria. This made them difficult to isolate until the following methods were adopted. Pieces of diseased root tissue, cut from the interior margin of a lesion, were surface sterilized in mercuric chloride (1/1000) for two minutes, soaked in sterile distilled water for at least one hour, and plated on potato-dextrose agar. Incubation of the plates at a low temperature, as suggested by Bisby, Timonin, and James (7), also greatly facilitated the isolation of these fungi. More isolates were obtained from material incubated at 10° C. than at a higher or lower temperature.

The isolates were provisionally identified by means of the key and descriptions published by Wollenweber (29). Most of these determinations were confirmed by Dr. Wollenweber, who kindly examined representative cultures.

C. Ehrenbergi predominated among the isolates, and was obtained from diseased roots taken from 25 of the 45 fields examined during the past two years. It was isolated more frequently from alfalfa than from sweet clover, but occurred on both hosts at widely separated points in Alberta. These districts included Brooks and Lethbridge in the southern brown soil zone, Edmonton and Lacombe in the black soil zone, and Athabasca and Beaverlodge in the northern gray wooded and transition soil zones. The fungus also occurred on diseased sweet clover roots sent from Scott and Saskatoon, in Saskatchewan. In addition, it was isolated from diseased roots of red clover and from decaying pods of string bean.

C. obtusisporum, *C. olidum*, and *C. radicicola* were occasionally isolated from alfalfa roots, but only the last was obtained from sweet clover roots. These species occurred on a small proportion of the diseased root samples, usually in association with *C. Ehrenbergi*. *C. obtusisporum* was also isolated from diseased raspberry roots.

C. Ehrenbergi was readily isolated from the soil of several alfalfa fields by the dilution plate method. The other three species occurred less commonly in these soils. The presence of *C. Ehrenbergi* in virgin soil was also demon-

strated. Sound tap roots of alfalfa were surface sterilized before being buried, during October, in virgin prairie sod at Edmonton, Alberta, and at Rossburn, Manitoba. In the following spring some of the roots buried at each location bore stromata of *C. Ehrenbergi*, and the fungus was isolated.

Cylindrocarpon spp. were not isolated when seed of several varieties and samples of alfalfa and sweet clover was plated on agar.

Infection Studies

Materials and Methods

Field-grown Grimm Alfalfa and Arctic sweet clover plants were used in all the general experiments, and were transplanted into boxes when required for greenhouse study. The field pathogenicity tests were made on dormant plants during the winter and on growing plants in the summer. In the winter experiments plants were inoculated in the late fall just prior to freeze-up. A small portion of oat hull inoculum was placed against each partially bared, but unwounded, tap root, after which the soil was replaced. Sterile oat hulls were placed against similar roots for checks, and all plants were left undisturbed until final notes were taken the following spring. A similar method was used in the summer experiments, except that the roots were wounded before inoculation, to promote infection. A thin flap of the outer tissue of the tap root was lifted with a sharp scalpel and a small fragment of mycelial inoculum was inserted beneath with a sterile wire hook. About four weeks later the plants were taken up.

In taking final notes each inoculated root was carefully examined and the degree of infection expressed by means of an arbitrary numerical rating on the following basis:

0 —No infection.
0.5—Slight trace of infection (surface tissues discolored).
1 —Trace infection (shallow lesions).
2–4—Light to medium infection (limited lesions of moderate depth).
5 —Medium infection (lesions extending about halfway through the tap root).
6–8—Medium to heavy infection (roots more than one-half rotted).
9 —Heavy infection (roots almost completely rotted).
10 —Plant dead.

Pathogenicity of *Cylindrocarpon* spp.

Thirty-five isolates of *C. Ehrenbergi* and a few isolates of the other species were tested for pathogenicity on roots of alfalfa and sweet clover in several different field experiments. Results obtained with representative isolates in two winter tests (Table I), and two summer tests (Table II), show that *C. Ehrenbergi* is decidedly more pathogenic than the other species studied. *C. obtusisporum* is slightly to moderately pathogenic in the early spring, but it produces only a trace of infection during the summer. *C. radicicola* is apparently only a weak wound parasite on roots of alfalfa and sweet clover, since

TABLE I

RELATIVE PATHOGENICITY OF SPECIES OF *Cylindrocarpon* AND ISOLATES OF *C. Ehrenbergi* ON ROOTS OF ALFALFA AND SWEET CLOVER. (WINTER TESTS 1935–36 AND 1936–37)

Species	Isolate		Alfalfa				Sweet Clover			
			Infection rating, %*			Rank†	Infection rating, %*			Rank†
	No.	Source	1935–36	1936–37	Av.		1935–36	1936–37	Av.	
C. Ehrenbergi	2	Alfalfa	24	5	14	10	22	23	22	13
C. Ehrenbergi	4	Sweet clover	18	5	11	11	61	60	60	5
C. Ehrenbergi	5	Sweet clover	35	16	25	6	33	91	62	4
C. Ehrenbergi	6	Alfalfa	47	49	48	1	12	73	42	9
C. Ehrenbergi	7	Alfalfa	23	27	25	6	37	40	38	12
C. Ehrenbergi	10	Sweet clover	18	47	32	5	30	91	60	5
C. Ehrenbergi	11	Sweet clover	17	27	22	8	64	92	78	1
C. Ehrenbergi	13	Bean pods	33	16	24	7	47	44	45	8
C. Ehrenbergi	14	Sweet clover	36	14	25	6	49	34	41	10
C. Ehrenbergi	15	Sweet clover	42	35	38	3	55	93	74	2
C. Ehrenbergi	18	Alfalfa	60	25	42	2	48	86	67	3
C. Ehrenbergi	22	Sweet clover	35	07	21	9	40	79	59	6
C. Ehrenbergi	23	Sweet clover	29	13	21	9	37	74	55	7
C. Ehrenbergi	28	Alfalfa	53	14	33	4	40	50	45	8
C. Ehrenbergi	30	Alfalfa	40	9	24	7	43	38	40	11
C. obtusisporum	26	Alfalfa	23	13	18		10	35	22	
C. obtusisporum	33	Alfalfa	31	11	21		17	10	13	
C. radicicola	17	Sweet clover	13	6	9		10	11	10	
C. radicicola	36	Alfalfa	14	5	9		1	12	6	
C. olidum	9	Alfalfa	7	2	4		0	0	0	
C. olidum	34	Alfalfa	5	6	5		0	0	0	
Check plants			6	1	3		0	0	0	

* *Average numerical rating of 15 plants in each test.*
† *Rank of 15 isolates of C. Ehrenbergi, based on average infection rating for two years.*

TABLE II

RELATIVE PATHOGENICITY OF SPECIES OF *Cylindrocarpon* AND ISOLATES OF *C. Ehrenbergi* ON ROOTS OF ALFALFA AND SWEET CLOVER. (SUMMER TESTS 1935 AND 1936)

Species	Isolate		Average infection rating, %					
			Alfalfa			Sweet clover		
	No.	Source	1935	1936	Av.	1935	1936	Av.
C. Ehrenbergi	2	Alfalfa	18	9	13	18	18	18
C. Ehrenbergi	4	Sweet clover	16	6	11	20	18	19
C. Ehrenbergi	5	Sweet clover	11	9	10	15	13	14
C. Ehrenbergi	6	Alfalfa	16	11	13	14	13	13
C. Ehrenbergi	7	Alfalfa	18	4	11	23	8	15
C. Ehrenbergi	10	Sweet clover	11	7	9	15	6	10
C. Ehrenbergi	11	Sweet clover	12	12	12	26	18	22
C. Ehrenbergi	13	Bean pods	10	11	11	8	12	10
C. Ehrenbergi	18	Alfalfa	12	10	11	11	2	6
C. Ehrenbergi	23	Sweet clover	14	13	13	25	26	25
C. obtusisporum	26	Alfalfa	10	17	13	8	12	10
C. obtusisporum	33	Alfalfa	5	7	6	8	9	8
C. radicicola	17	Sweet clover	8	5	6	8	2	5
C. radicicola	36	Alfalfa	6	2	4	2	0	1
C. olidum	9	Alfalfa	5	0	2	3	0	1
C. olidum	34	Alfalfa	7	0	3	3	1	2
Check plants			6	0	3	5	0	2

it seldom causes more than a slight discoloration of the tissues. None of the several isolates of *C. olidum* tested during the past three years have given definite evidence of pathogenicity.

Pathogenicity of Isolates of *C. Ehrenbergi*

It is evident from the data in Table I that isolates of *C. Ehrenbergi* differ in degree of pathogenicity. Some isolates are highly virulent on both alfalfa and sweet clover, while others produce relatively light infection. On the other hand, certain isolates which are highly pathogenic on sweet clover rank among those least pathogenic on alfalfa, and *vice versa*. Isolate 6, obtained from alfalfa, and Isolate 11, from sweet clover, are particularly virulent on their respective host plants, but otherwise there is no evidence of host specialization among the isolates. An isolate from bean pods attacked both hosts with moderate severity. Marked differences in pathogenicity are not evident in the summer tests (Table II), owing to the greatly reduced severity of infection.

Varietal and Host Range Tests

Tests of varietal reaction to *C. Ehrenbergi* are still in a preliminary stage, but some indications of the potentialities of the commonly grown varieties of alfalfa and sweet clover have been obtained. In three winter tests (1934–35 to 1936–37), the soil was infested with the pathogen at seeding time, as in varietal tests with *Sclerotinia* sp. (24). Since this method gave a relatively low degree of infection with *C. Ehrenbergi*, roots of plants of each variety were also directly inoculated in the fall of 1936. The results obtained in these experiments are given in Table III.

TABLE III

Reaction of varieties of alfalfa and sweet clover to *Cylindrocarpon Ehrenbergi* in winter tests

Variety	Average infection rating, %*				
	Soil infested				Roots inoculated
	1934–35	1935–36	1936–37	Av.	1936–37
Alfalfa					
Medicago falcata	1	3	4	3	11
Ladak	11	6	6	8	13
Grimm	7	14	4	8	21
Cossack	10	9	7	9	27
Baltic	11	9	6	9	20
Hardistan	20	17	8	15	30
Sweet clover					
Yellow blossom	22	16	18	19	69
Arctic	24	18	18	20	62
White blossom	28	14	19	20	55
Alpha No. 1	18	24	27	23	69
Zouave	27	26	22	25	65
Grundy County	31	29	21	27	57
Albotrea	29	30	29	29	75

* *Average infection rating of 60 plants of each variety in each test.*

Medicago falcata (yellow-flowered alfalfa) appears to be relatively resistant to attack by *C. Ehrenbergi*. In previous studies (24), this species of alfalfa proved markedly resistant to attack by *Sclerotinia* sp. Hardistan alfalfa (*M. sativa*) had consistently the highest infection rating of the varieties tested. The variegated alfalfa varieties (*M. media*) gave a reaction intermediate between that of *M. falcata* and Hardistan.

All varieties of sweet clover tested were attacked with approximately equal severity by *C. Ehrenbergi*. Albotrea appeared slightly more susceptible than the other varieties, but further study is required. There were no clear-cut differences in reaction between varieties of *Melilotus alba* and *M. officinalis*, such as were obtained in studies with *Sclerotinia* sp. (24).

C. Ehrenbergi has also proved pathogenic on roots of *Trifolium* spp. In two winter tests it caused moderate to heavy infection on red clover, and light to moderate infection on alsike clover and white Dutch clover. At the same time *C. obtusisporum* produced light infection on the roots of these three hosts. A trace to light infection occurred, in all cases, under summer conditions.

C. Ehrenbergi caused slight rotting of wounded roots of turnip and carrot in a summer field experiment, but did not attack roots of beet or parsnip. It was also non-pathogenic to seedlings of wheat, oats and barley in the greenhouse.

Factors Influencing Infection

Periodic observations on alfalfa and sweet clover roots inoculated with *C. Ehrenbergi* in the late fall showed that infection did not occur until the soil started to thaw out in April, but then proceeded very rapidly. However, relatively light infection occurred when the roots were inoculated shortly after the soil thawed out. In another experiment, dormant plants taken from partially frozen soil were inoculated, transplanted into boxes, and held at temperatures of 1.5°, 17°, and 21° C. Slightly greater infection was obtained at 1.5° C. than at the other temperatures, but in no case were the roots severely attacked. The plants were apparently past their most susceptible stage when the early spring inoculations were made. When the progress of infection during the spring was studied by inoculating plants at weekly intervals in the field, more infection occurred during April than later in the season.

Isolates of *C. Ehrenbergi* appear to vary with regard to the influence of temperature on infection. Isolate 23 produced light to moderate infection on roots of sweet clover growing in soil temperature tanks held at 18° and 21° C., but three other isolates were non-pathogenic at these temperatures. None of the isolates caused infection at temperatures of 24° and 27° C. Isolate 23 was also one of the most pathogenic of the isolates under the relatively high temperature conditions of summer (Table II), but one of the least pathogenic in the early spring (Table I).

Seedlings and young plants of alfalfa and sweet clover were less susceptible than older plants to attack by *C. Ehrenbergi*. In summer tests the infection rating for two-months-old plants was consistently less than for 14-months-old plants. With alfalfa, the roots of plants three and four years old were more severely attacked in the early spring than the roots of plants one and two years old.

Pathological Anatomy

The phenomena attending penetration and invasion of alfalfa and sweet clover roots by *C. Ehrenbergi* were studied for both early spring and summer infection. Roots were taken up for histological examination at various stages of disease development. Small pieces of each root were thoroughly fixed in modified Bouin's fluid, washed in four changes of 50% ethyl alcohol, and dehydrated in the tertiary butyl alcohol series described by Johansen (16). After infiltration with paraffin, the material was softened in water and cut, on a sliding microtome, into sections 12μ thick. The Thionin-Orange G staining method described by Stoughton (25) gave very satisfactory differentiation of the fungus mycelium in the host tissues.

Wounding of the roots usually facilitates the entrance of *C. Ehrenbergi*, but is not necessary for successful infection. In the absence of wounds, the pathogen can enter the roots by three different avenues, namely: through the tissues at the base of branch roots, through lenticels, or by direct penetration of the cork covering. Infection occurs most commonly at the base of a branch root, which is a very vulnerable point, since it is protected only by a thin, and often broken layer of cork. The hyphae start to grow in the loose tissues around the base of a branch root, and soon rot it off and surge into the underlying tissues (Plate I, A). Lenticels are penetrated in a similar manner. Direct penetration appears to be less common, but has been frequently observed when a considerable mass of inoculum is in contact with the uninjured root. The hyphae mass up and push their way between the cork cells in an apparently mechanical manner (Plate I, B). The cork cells remain intact at first, but are soon ruptured and filled with the massed hyphae. This process is repeated in the deeper-lying cells, until the entire cork layer is penetrated. *C. Ehrenbergi* thus appears to effect penetration chiefly by mechanical means, in a manner similar to that described by Peltier and King (17) for direct penetration of alfalfa roots by *Ozonium omnivorum*. Previous studies on the invasion of alfalfa and sweet clover roots by *Sclerotinia* sp. and *Plenodomus Meliloti* (8) indicated that chemical action played a part in the penetration of cork layers by those pathogens.

After entering the root, the hyphae of *C. Ehrenbergi* develop rapidly in the phloem parenchyma. They progress singly, or more commonly in wefts or masses, through and between the cells and in all directions. Unless infection is severe, the hyphae are confined mainly to the parenchymatous tissues, and less commonly invade the phloem, cambium, and xylem. They seldom develop in the vessels. During summer, wound cork layers are often formed in advance of hyphal invasion, but, as with *Sclerotinia* sp. and *Plenodomus*

Meliloti (8), they do not appear effective in permanently checking the progress of the pathogen (Plate I, C). Hyphal advance ceases, however, in about 20 days after inoculation, under summer conditions. A distinct line of demarcation is formed between the diseased and healthy tissues. This border is of varying width and consists of disorganized cells filled with a dark-staining material. Apart from this, *C. Ehrenbergi* does not appear to exert any marked chemical action on the tissues in advance of hyphal invasion.

When infection has reached an advanced stage, *C. Ehrenbergi* produces stromata in irregular layers or sclerotia-like masses on the surface of the rotted tissues (Plate I, D). In the early stages of stromatal formation the hyphae mass up and fuse into small, soft bodies, which gradually increase in size and become fairly hard and brittle. Mature stromata are erumpent, or partly embedded in the disorganized cork layer, and they consist of closely packed hyphal elements united in a pseudo-parenchymatous tissue (Plate I, E).

The Pathogens

Literature Review of the Genus *Cylindrocarpon*

Taxonomy. This relatively new genus was established by Wollenweber in 1913 (26). It belongs to the family Tuberculariaceae of the Fungi Imperfecti and contains fungi with cylindrical-clavate conidia, which were previously included in the genera *Fusarium*, *Ramularia*, *Fusidium*, *Fusisporium*, *Septocylindrium*, and *Atractium*. The species with known ascigerous stages are conidial forms of *Nectria* spp. Chlamydospore-producing forms originally placed in the genus *Ramularia* (26) were later transferred to *Cylindrocarpon* (27, 28). These two genera are distinguished by their manner of conidial abstriction (29). In *Cylindrocarpon*, as in *Fusarium*, conidia are formed in basipetal succession, with the oldest conidium uppermost. In *Ramularia*, however, each conidium arises acropetally as a bud-like outgrowth from an older conidium. Conidia of *Cylindrocarpon* differ from those of *Fusarium* in being cylindrical, less dorsiventral, and apedicellate, with a rounded apex. In many other respects the two genera are very similar.

Most of the species of *Cylindrocarpon* were fully described by Wollenweber (29) in 1928. They are differentiated on characteristics similar to those employed for *Fusarium*. The section Ditissima contains all the known conidial stages of members of the sub-genus *Coryneconnectria* and other species which do not form chlamydospores. Ten species and 11 varieties have been described in this section (18, 29). Species which produce chlamydospores are placed in the section Chlamydospora, which now contains 11 species and one variety (18, 29, 30). Members of this section are also characterized by relatively small, usually straight conidia, which often have basal papillae. The ascigerous stages belong to the sub-genus *Neonectriae*, but are only known for two of the species.

The four species studied in the present investigation belong to the section Chlamydospora. *C. Ehrenbergi* Wr. was first described as *Fusarium candidum*

by Ehrenberg in 1818. Other synonyms are *Ramularia candida* (Ehr.) Wr., and *Fusarium uniseptatum* v. Hoehnel. The ascigerous stage, *Neonectria caespitosae* (Fuck.) Wr., which was found on bark of *Ulmus* and dead roots of *Betula* in Germany (29), has not been observed during the present study.

Synonyms for the other species studied are as follows:

C. obtusisporum (Cke. & Hark.) Wr.—*Fusarium obtusisporum* Cke. & Hark., *Ramularia obtusispora* (Cke. & Hark.) Wr., *R. anchusae* Wr.

C. radicicola Wr.—*Fusarium polymorphum* Marchal, *Ramularia macrospora* Wr.

C. olidum Wr.—*Fusarium solani* Sacc., *Ramularia olida* Wr.

Distribution and Parasitism of the Species. Species of *Cylindrocarpon* have been isolated from decaying roots, tubers, bulbs, stems, branches, and fruit of many plants (29). Those of most importance on the aboveground parts of plants are the conidial stages of *Nectria* spp. European canker of pears, apples, and other trees, caused by *N. galligena* (*C. Mali*), is a destructive disease in many countries (2, 33). Other canker-producing species were studied by Richter (20), in Europe, and Zeller (34), in Oregon. Ehrlich (10) made a comprehensive study of the beech bark disease caused by *N. coccineae* (*C. candidum*). Wollenweber and Hochapfel (32) recently found that several species of *Cylindrocarpon* occurring on rotted fruits were parasitic on fruits of apple, pear and tomato.

Species of the section Chlamydospora occur quite commonly on the underground parts of plants (29), but their parasitism has received relatively little attention. One of the best known species, *C. radicicola*, causes a destructive bulb rot and root rot of daffodils, narcissus, hyacinth, cyclamen, and other bulbous plants in England and Europe (3, 11, 31). Berkeley and Lauder-Thomson (5) found this species to be the most virulent of the fungi isolated from the "black lesion" type of strawberry root rot in England. It has been also found associated with the root-rot complexes of strawberry and raspberry in Ontario (4, 13). Jenkins (15) reported that *Ramularia macrospora* (*C. radicicola*) caused a disease resembling crown canker on greenhouse roses in the United States. Hodges (14) isolated *C. radicicola*, *C. radicicola* var. *violaceum*, and *C. didymum* from sugar beets, but found that they caused little or no damage to that host. Root rot of ginseng in Ontario, studied by Hildebrand (12), was caused by *Cylindrocarpon*-like fungi which were referred to the genus *Ramularia*.

The occurrence of *Cylindrocarpon* spp. in soils is further evidence of their wide distribution. Wollenweber (29) reported *C. radicicola*, *C. didymum* and *C. Magnusianum* from soil. Bisby, James and Timonin (6) isolated the following forms from Manitoba soils: *C. macrosporum*, *C. candidum*, *C. candidum* var. *majus*, *C. heteronemum*, and *C. didymum*. In a later study (7), these workers found the above species of fairly common occurrence, especially in the sub-surface horizons of the soil. Reinking (19) recently found *C. olidum*

and two new varieties of this species, also *C. curvatum*, and *C. radicicola* in soils of banana plantations in South America. The relative prevalence of these species varied at different depths in the soil.

Cylindrocarpon spp. have apparently not been previously reported on alfalfa, sweet clover, or other legume forage crops. The four species isolated from these hosts during the present investigation have been incidentally reported on various plants, but, with the exception of *C. radicicola*, discussed above, little is known concerning their parasitism. *C. Ehrenbergi* was mentioned by Wollenweber (26) as a possible wound parasite on partially decayed roots of carrots and other plants. Later (29), he listed it as occurring in Europe on roots of *Daucus;* tubers of *Solanum tuberosum;* bark of *Fagus;* branches of *Betula;* stalks of *Brassica*, *Helianthus*, and *Lupinus;* fruits of *Aesculus*, *Juglans*, and *Pirus;* and seedlings of *Pinus*. *C. obtusisporum* has been found on rotting seed of *Triticum vulgare*, *Phytophthora*-rotted potato tubers, branches of *Tilia* and *Acaciae*, bark of *Pirus*, and wood of *Ribes* (29). *C. olidum* was reported by Wollenweber (29) on decayed potato tubers in Europe, and by Anliker (1) on diseased rye seedlings in Switzerland.

Description of the Isolates

General Characteristics. The isolates of *C. Ehrenbergi* obtained from roots of alfalfa and sweet clover correspond fairly closely to Wollenweber's Latin diagnosis of the species (29). Most strains produce white mycelium in a compact, cottony, slow-growing colony, which is white, or some shade of orange, salmon, or vinaceous, depending upon the color of the basal stromatal layer (Plate I, F). Characteristic sclerotia-like stromata, resembling those formed on infected roots (Plate I, D), are often produced on agar. They are scattered throughout the colony, or aggregated in masses or concentric rings, and vary in color from yellow, orange, salmon, cinnamon to brown, with occasionally an olive-green tinge. Conidia are usually absent, or sparingly borne on the mycelium, in cultures less than one month old. They are produced successively at the tip of a conidiophore, and often adhere, side by side, to form a false head. In older cultures, creamy white shiny masses or layers of conidia are borne in sporodochia or pionnotes which develop from stromata or on the mycelium. The conidia are cylindrical and straight, or slightly curved, with ellipsoidal to conical ends (Fig. 2, A-B). They are typically one-septate, with an average size range of 20–27 $\times$ 3.3–3.9μ, in the strains studied (Table IV). Pluri-septate conidia were not observed and non-septate forms were rare in most of the cultures. Chlamydospores are usually scarce. They are spherical to ovate, intercalary, single or in short chains, and average about 10 $\times$ 8μ in size (Fig. 2, C).

Isolates of *C. obtusisporum* can be readily distinguished from those of *C. Ehrenbergi*. Short, dense, white or pale yellow mycelium is produced on a dark brown stromatal layer, forming a compact, zoned, yellowish-brown colony (Plate I, F). Cylindrical-clavate, 0- to 3-septate conidia, with obtuse-conical ends (Fig. 2, D), are borne on the mycelium and, more rarely,

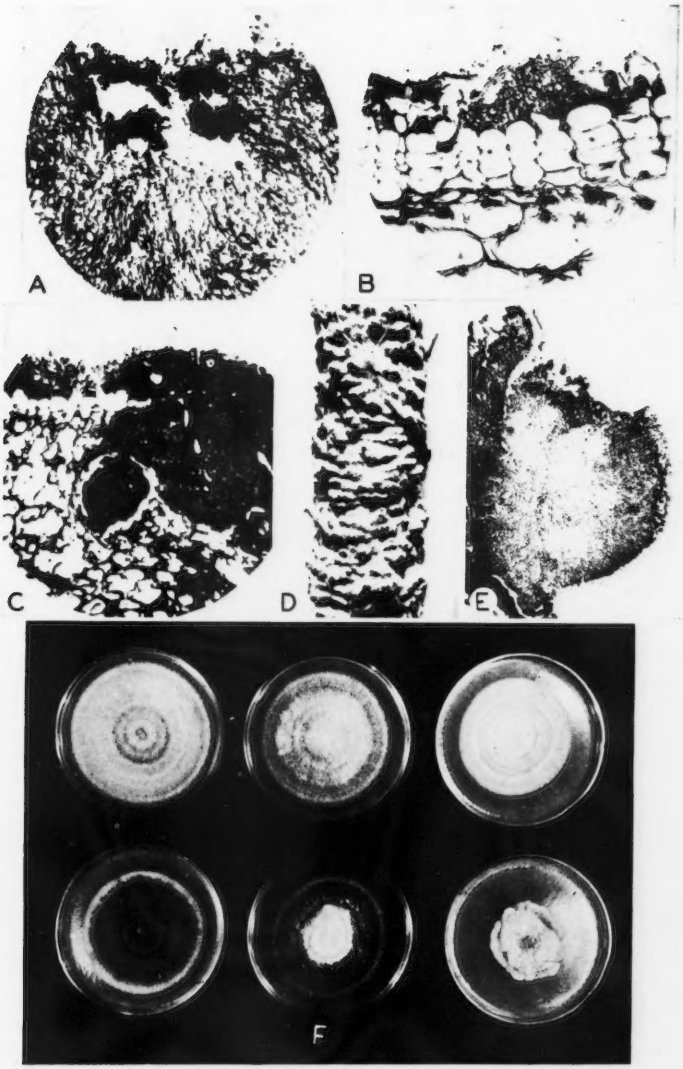

PLATE I. A-E. *Invasion and development of Cylindrocarpon Ehrenbergi in roots of alfalfa and sweet clover.* A. *Hyphae entering a tap root through the base of a branch root which has been rotted off.* ×*85.* B. *Massed hyphae effecting direct penetration by pushing between the cork cells of the outer protective layer of the root.* ×*330.* C. *Ineffective wound cork layers (X) formed around the invading hyphal mass.* ×*130.* D. *Numerous stromata aggregated on the surface of an infected root.* ×*3.* E. *Longitudinal section of a single stroma which is partly embedded in a diseased root.* ×*85.* F. *Ten-day-old colonies of Cylindrocarpon* spp. *on potato-dextrose agar. Left to right: Top row, C. Ehrenbergi. Isolates 7, 4, and 30. Bottom row: C. obtusisporum, C. radicicola, and C. olidum.*

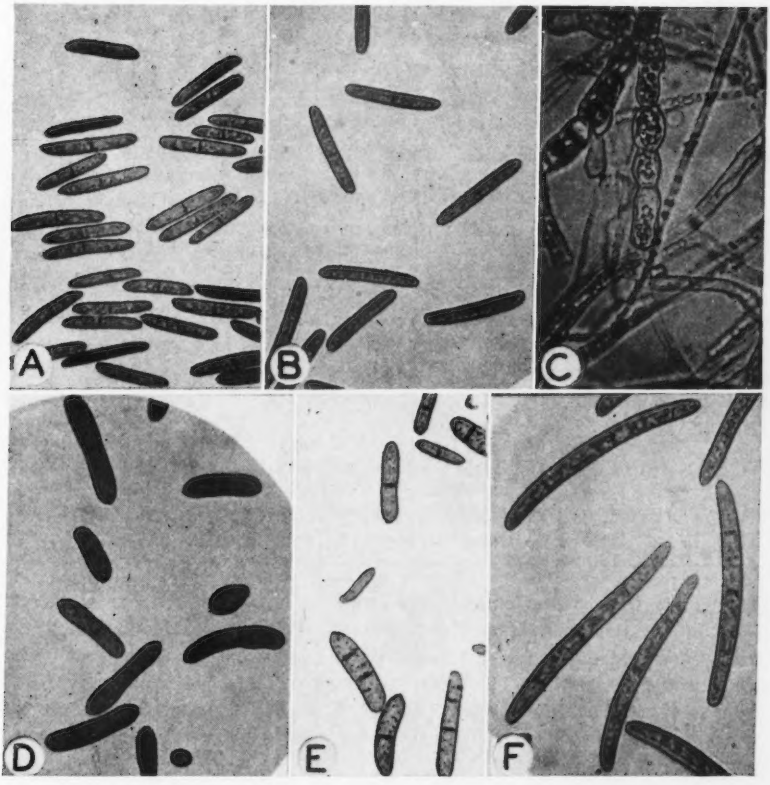

FIG. 2. *Conidia and chlamydospores of Cylindrocarpon spp. from six-weeks-old colonies grown on potato dextrose agar. × 475. (Lightly stained.)* A-C. *C. Ehrenbergi. Conidia of Isolates 4 and 6, and chlamydospores of Isolate 11, respectively.* D-F. *Conidia of C. obtusisporum, C. radicicola, and C. olidum, respectively.*

in sporodochia or pionnotes. The size of the prevailing 0- and 1-septate forms (150 of each measured), in six-weeks-old cultures on potato-dextrose agar, was as follows:

0-septate, average 12.9 × 4.7μ; majority 9.1–18.2 × 4.2–5.7μ; total range 7.6–19.8 × 3.4–7.0μ.

1-septate, average 21.1 × 5.0μ; majority 15.2–27.4 × 4.6–5.7μ; total range 9.8–33.4 × 4.2–6.1μ.

Chlamydospores are rare and not well differentiated from bulbous swellings on the mycelium.

C. radicicola has relatively sparse, pale yellow to light brown aerial mycelium produced on a stromatal layer which sometimes has a purplish tinge (Plate I,F).

TABLE IV

CHARACTERISTICS OF GROUPED ISOLATES OF *Cylindrocarpon Ehrenbergi*

Group	No. of isolates	Colony		Conidia			Stromata	Chlamydospores
		Growth rate	Color	Production	Type	Av. size, (μ)		
A	5	Slow	Vinaceous to ferruginous	Abundant	Long, slender, 1-sept.	25–27 × 3.4	Scarce	Scarce
B	10	Medium	Varied but mostly salmon	Scarce	Fairly short 1-sept.	21–24 × 3.4–3.9	Abundant	Scarce
C	3	Medium	White to vinaceous	Medium	Short, 1-sept.	18–22 × 3.4–3.6	Medium	Scarce
D	7	Rapid	Pinkish cinnamon	Abundant	Variable, 0 and 1-sept.	20–22 × 3.3–3.7	Variable	Scarce
E	2	Medium	White	Scarce	Fairly short 1-sept.	20–23 × 3.4–3.5	Absent	Scarce
F	1	Medium	Cinnamon	Scarce	Short 1-sept.	21.7 × 3.5	Medium	Abundant

The conidia, formed on the mycelium or in pionnotes, are cylindrical, mostly 1- to 2-septate, and straight or slightly curved, with ellipsoidal ends (Fig. 2, E). In six-weeks-old cultures on potato-dextrose agar, 150 of the predominant 1-septate conidia measured as follows: average 23.4 × 5.4μ; majority 18.2–28.9 × 4.6–6.1μ; total range 13.7–31.9 × 3.8–6.5μ. Chlamydospores are abundantly produced. They are brown, nearly spherical, intercalary, and average about 11μ in diameter.

C. olidum is most readily recognized by the strong earthy odor produced in pure culture. The abundant, white to light brown mycelium has raised patches and tufts, formed in an irregular zonal manner, on a light brown stromatal layer (Plate I, F). Conidia are produced in white to honey-colored sporodochial masses. They are large, distinctly curved, and mostly 3-septate, with ellipsoidal ends (Fig. 2, F). In six-weeks-old cultures on potato-dextrose agar, 150 three-septate conidia of one isolate measured as follows: average 54.6 × 6.1μ; majority 48.6–60.8 × 5.7–6.5μ; total range 38.6–68.3 × 4.6–6.8μ. Other isolates have slightly smaller or larger conidia. The fairly numerous chlamydospores are hyaline, 1- to 2-celled, verrucose, mostly intercalary, and average about 12 × 7μ in size.

Description of C. Ehrenbergi Strains. Isolates of *C. Ehrenbergi* were found to differ greatly in morphological, cultural, and physiological characteristics, as well as in pathogenicity. A detailed study was made of 28 isolates in an attempt to determine their range of variability. These isolates have been grouped in Table IV for purposes of comparison.

Some isolates grow much more rapidly than others at room temperature (Plate I, F), but the differences are less marked at low temperatures. Color of the mycelium and substratum varies greatly in different strains, and is also influenced by the kind of medium, temperature and other conditions. The colony colors listed in Table IV are based on Ridgway (21), and are those which predominated under a wide range of conditions. Marked and consistent differences occur in the rapidity and abundance of conidial formation. Some isolates (Groups A and D) produce creamy white masses of conidia in four to six weeks, while other isolates (Group B) never produce them in masses, and only sparingly on the mycelium. These isolates also differ greatly in the number and type of stromata produced.

Another striking point of difference between isolates is in conidial size and shape. The range of size for representative isolates, grown under identical conditions, is given in Table V. There is a marked contrast between the long, relatively slender conidia of isolates in Group A and the short conidia

TABLE V

SIZE IN MICRONS OF CONIDIA FROM DIFFERENT ISOLATES OF *Cylindrocarpon Ehrenbergi**

Group	Isolate No.	Type of spore	Average size	Size of majority (90%)	Total range
A	4	1-sept.	26.0×3.4	23.5–29.7×3.0–3.4	20.6–32.7×2.6–3.7
	14	1-sept.	27.0×3.4	24.1–29.2×3.0–3.4	21.0–32.7×3.0–3.8
B	6	1-sept.	22.0×3.4	19.8–24.1×3.0–3.8	17.2–25.8×3.0–4.3
	18	1-sept.	21.2×3.9	20.6–23.3×3.8–4.3	18.9–24.1×3.4–4.7
C	10	1-sept.	21.0×3.4	18.9–24.1×3.4–3.8	16.4–25.0×3.0–4.3
D	23	1-sept.	22.1×3.3	20.6–24.1×3.0–3.4	17.2–25.8×3.0–3.8
	20	1-sept.	20.3×3.7	17.2–23.3×3.4–3.8	14.7–25.0×3.0–4.3
	20	0-sept.	11.2×3.2	9.1–12.9×3.0–3.4	7.8–15.2×2.6–4.3
F	11	1-sept.	22.1×3.3	20.6–24.1×3.0–3.4	17.2–25.8×3.0–3.8
	11	Chlamydospores	10.6×7.8	8.6–12.0×6.9–9.5	6.9–15.5×5.2–13.8

* *Measurement of 150 spores of each isolate from two-months-old cultures on potato dextrose agar.*

of isolates in Group B (Fig. 2, A-B). The former type is consistently associated with the slow-growing, dark-colored colony characteristic of Group A. Isolates in the other groups are more variable and, in general, do not show any correlation between morphological and cultural characteristics.

Certain isolates possess distinctive characteristics not found in any of the other strains studied. For example, Isolate 20 produces approximately 33% small, non-septate conidia, which usually represent less than 1% of the conidia of other isolates. Chlamydospores are sparingly produced by most isolates, but occur abundantly under all conditions in cultures of Isolate 11. Two isolates (Group E) produce white sparse mycelium, and no stromata (Plate I, F),

but otherwise resemble the isolates of Group B. This type may possibly arise as a variant in nature, since it occasionally appears as a sector in pure culture.

There is apparently no correlation between the morphological and cultural characteristics of an isolate and its virulence, for isolates in each of the groups described above were among those most pathogenic on alfalfa, and also among those most pathogenic on sweet clover (Table I).

Cultural Studies

Influence of Nutrients. Potato-dextrose agar supported good growth of *Cylindrocarpon* spp., and was used in all the general experiments. *C. Ehrenbergi* was tested on a variety of natural and synthetic media. Potato-dextrose agar gave the best results, but the fungus also grew well on Czapek's synthetic malt extract, and soil extract agars, and on agar media prepared from the expressed root juices of alfalfa and sweet clover. Media unsuitable for mycelial growth were corn meal, bean pod, prune, oat hull, Dox's inorganic salt, and Molisch's salt peptone agars.

Dextrose and peptone were added in varying concentrations to agar media. Moderate amounts of dextrose stimulated mycelial growth of *C. Ehrenbergi*, and usually favored stromatal production. Conidial formation was definitely retarded on media containing more than 2% dextrose. Peptone stimulated mycelial growth for the first few days, but hastened staling action in later growth. It had a slight stimulating effect on stromatal and conidial formation. Other studies also indicated the importance of the influence of the C/N ratio on growth and reproduction of the fungus.

On ordinary media most strains of *C. Ehrenbergi* produce very few stromata or conidia until the cultures are at least one month old. A modified Molisch's salt-peptone agar, however, induced good stromatal and conidial formation within 10 to 15 days. Stromata and conidia also formed quite rapidly on steam-sterilized pieces of alfalfa root in test tubes. Similarly prepared pieces of roots of sweet clover, red clover, and alsike clover did not provide favorable media for their development.

Influence of Temperature. The relation of temperature to growth in pure culture of *C. Ehrenbergi* and *C. obtusisporum* was studied in several different experiments. Uniform circular pieces of inoculum were transferred to plates of potato-dextrose agar, which were immediately incubated in quadruplicate at controlled temperatures ranging from 1° to 33° C. Representative results obtained with five isolates of *C. Ehrenbergi* and one isolate of *C. obtusisporum* are given in Table VI.

All five isolates of *C. Ehrenbergi* grew well at temperatures ranging from 14° to 27° C., and were inhibited, but not killed, at 33° C. Isolates 4, 20, and 31, however, grew best at about 20° C., while Isolates 11 and 23 had a somewhat higher optimum temperature. These temperature relations were

TABLE VI

GROWTH OF FIVE ISOLATES OF *Cylindrocarpon Ehrenbergi* AND ONE ISOLATE OF *C. obtusisporum* AFTER INCUBATION FOR SEVEN DAYS AT TEMPERATURES RANGING FROM 1° TO 33° C.

Species	Isolate No.	Average diameter of colonies in mm. at different temperatures									
		1°	5°	9°	14°	17°	20°	24°	27°	30°	33°
C. Ehrenbergi	4	5	8	12	19	21	23	22	20	8	0
C. Ehrenbergi	20	5	10	17	30	35	38	37	19	11	0
C. Ehrenbergi	31	5	10	14	23	31	32	27	20	6	0
C. Ehrenbergi	11	5	9	15	26	30	32	33	18	7	0
C. Ehrenbergi	23	5	10	15	21	26	29	32	29	11	0
C. obtusisporum	26	0	5	10	25	31	31	16	13	0	0

confirmed in another experiment with some of the isolates (Fig. 3). The relatively high temperature relation of Isolate 23 may explain its ability, during summer, to attack roots of alfalfa and sweet clover more severely than most other isolates (Table II).

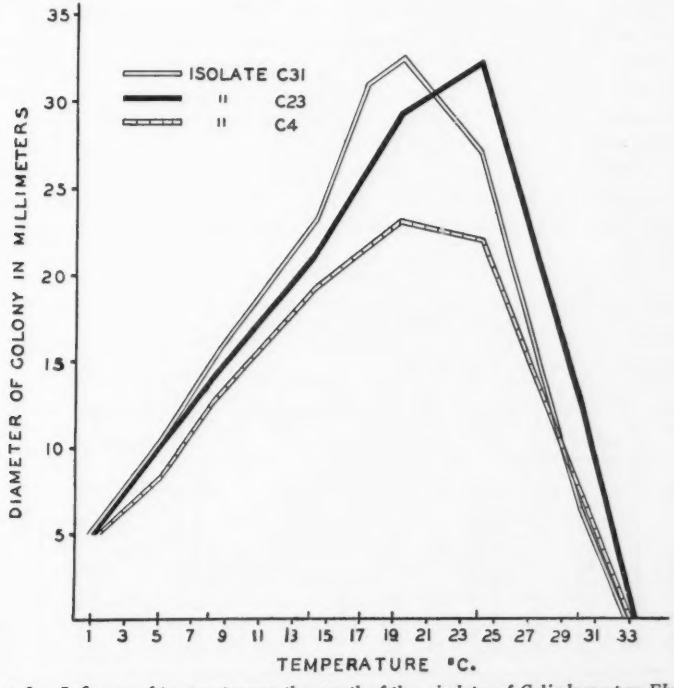

FIG. 3. *Influence of temperature on the growth of three isolates of Cylindrocarpon Ehrenbergi on potato-dextrose agar. Incubated seven days.*

C. obtusisporum had a relatively narrow temperature range for growth, with an optimum at 17° to 20° C. It was greatly retarded at the higher temperatures, and failed to grow at 30° C.

The low-temperature relations of the isolates were of special interest in this investigation, owing to their association with winter injury of alfalfa and sweet clover. *C. Ehrenbergi* started growth on frozen agar three weeks after freshly transferred cultures of several isolates had been placed in a freezing chamber at −2° C. Isolates of *C. obtusisporum*, *C. radicicola*, and *C. olidum* did not grow at this temperature. At 1° and 5° C. all isolates of *C. Ehrenbergi* developed at an approximately equal rate (Table VI). At 1° C. *C. obtusisporum* and *C. radicicola* started growth in 10 and 14 days, respectively. *C. olidum* failed to grow at 1° C., and developed very slowly at temperatures below 10° C.

Influence of Hydrogen Ion Concentration. Isolates of *C. Ehrenbergi* were grown on buffered potato-dextrose and Czapek's liquid and agar media adjusted to a wide range of hydrogen ion concentration. A large batch of each medium was made up with less than the normal amount of water, and divided into lots for adjustment. Each lot was sterilized and then adjusted to the required reaction by adding sterilized hydrochloric acid or sodium hydroxide. If required, sterile water was added to bring each lot up to normal concentration. pH determinations were made with a potentiometer and quinhydrone, and were checked by the colorimetric method. The agar media were held at 40° C. for adjustment and determination of pH, after which uniform amounts were poured into sterile plates. With the solutions, each lot was allowed to stand for 24 hr. before final adjustment, and then 50-cc. portions were pipetted into sterile flasks. Uniform pieces of inoculum were transferred to the plates and flasks, which were incubated at room temperature. Representative results obtained with one isolate are given in Table VII.

TABLE VII

INFLUENCE OF THE HYDROGEN ION CONCENTRATION OF AGAR AND LIQUID MEDIA ON GROWTH OF *Cylindrocarpon Ehrenbergi* (Isolate 4)

Potato dextrose agar		Czapek's agar		Czapek's solution			
Initial pH	Diameter* of colonies	Initial pH	Diameter* of colonies	Initial pH	Final pH	Final pH of control	Dry weight of mycelium†
2.1	0						
3.1	0	2.8	8	3.0	3.1	3.0	0
4.1	22	4.0	28	3.9	4.6	3.8	75
4.7	27	4.6	29	4.6	5.2	4.5	154
5.2	30	5.1	28	5.2	5.2	5.2	125
5.9	31	5.8	27	5.8	5.4	5.8	127
6.3	31	6.2	29	6.4	6.0	6.4	129
7.0	31	6.9	28	7.0	6.2	6.9	133
7.6	31	7.7	25	7.8	6.6	7.6	262
8.4	30	8.4	24	8.4	7.0	8.2	183
9.4	26	9.5	21	9.7	7.8	9.5	66

* *Average diameter, in millimeters, of four colonies, after seven days' incubation.*
† *Average dry weight, in milligrams, of four colonies, after two weeks' incubation.*

C. Ehrenbergi grew well at hydrogen ion concentrations ranging from 4.0 to 9.5. The optimum reaction for growth varied with the medium employed. On potato-dextrose agar, the best growth occurred at pH 5.9 to 7.6, and there was none at pH values lower than 4.1. The optimum was also poorly defined on Czapek's agar, but the fungus was more tolerant to the acid reactions and grew at pH 2.8. More conclusive results were obtained in Czapek's solution, where two distinct optimum points for growth occurred at pH values of 4.6 and 7.8. Less growth at pH 5.2 to 6.4 indicated an isoelectric point in this region (22). Growth of the fungus resulted in the medium becoming more alkaline when the initial pH values were below 5.2, and more acid when the initial values were above this point. Only slight pH changes occurred in the corresponding control solutions where the fungus was not grown.

Spore Germination of *C. Ehrenbergi*

Conidia of *C. Ehrenbergi* were germinated at different temperatures and in solutions of varying hydrogen ion concentration. Three drops of a uniform suspension of mature conidia were distributed on a chemically clean, sterile, microscope slide. The slide was placed on a piece of bent glass tubing in an inverted Petri dish, which was sealed with water. Under favorable conditions, the conidia germinated within 15 hr., by producing a germ tube at the end of each cell. The percentage of spore germination for each treatment was calculated from a count of approximately 100 spores in each of the triplicate drops.

Spore suspensions of Isolate 4 in potato-dextrose solution (pH 7.0) were incubated at temperatures ranging from 2° to 33° C. In 15 hr., 97% of the conidia had germinated at 20° C., 93% at 17° C., 75% at 13° and 25° C., and 52% at 28° C. Germination was inhibited at higher temperatures. At low temperatures the spores germinated slowly, but even at 2° C. most of them produced germ tubes within four days.

Conidia of Isolate 4 were germinated in potato-dextrose solutions with pH values ranging from 2.0 to 8.6. The results obtained with duplicate slides incubated at 18° and 24° C. are given in the first columns of Table VIII. The spores germinated readily at pH values ranging from 3.4 to 8.6. At the lower pH values, higher germination occurred at 18° C., than at 24° C. It started in four days at pH 3.0 and 2.4, but was inhibited at pH 2.0.

At both temperatures, optimum points for spore germination occurred at pH 4.0 to 4.4, and at pH 7.2 to 7.6, with a distinct minimum at pH 5.2. This minimum point was more marked at 18° than at 24° C. It was more accurately determined in a second experiment where solutions with a narrower range of pH values were employed (Table VIII). The minimum or apparent isoelectric point for spore germination occurred at pH 5.1, as evidenced by a lower germination and by the relatively weak germ tube growth of conidia which had germinated. Similar results were obtained with other isolates. Since a similar, but less well defined, minimum point occurred in the growth studies, it is concluded that the isoelectric point for *C. Ehrenbergi* lies at approximately pH 5.1.

TABLE VIII

INFLUENCE OF HYDROGEN ION CONCENTRATION AND TEMPERATURE ON SPORE GERMINATION OF *Cylindrocarpon Ehrenbergi* IN POTATO DEXTROSE SOLUTION

First experiment			Second experiment		
Initial pH	Per cent germination*		Initial pH	Per cent germination, 20° C.	Germ tube† length, 20° C.
	18° C.	24° C.			
2.0	0	0			
2.4	0	0			
3.0	0	0	4.7	97	
3.4	84	46	4.8	95	
4.0	100	99	4.9	88	18.6
4.4	99	98	5.0	75	9.8
4.8	74	87	5.1	57	3.5
5.2	28	70	5.2	89	12.0
5.6	85	94	5.3	92	22.1
6.0	86	96	5.4	89	
6.4	82	96			
6.8	75	98			
7.2	94	100			
7.6	96	98			
8.0	88	93			
8.6	87	90			

* *Per cent germination in 15 hr.*
† *Average length in microns of 100 germ tubes in 15 hr.*

Discussion

The foregoing results indicate that *C. Ehrenbergi* is probably one of the most important of the fungi associated with early spring injury of alfalfa and sweet clover in Alberta. In fact, it may be even more prevalent than *Plenodomus Meliloti* and *Sclerotinia* sp. It has been isolated from a large proportion of the diseased root samples collected from widely separated points throughout the province, and all isolates studied have proved pathogenic on roots of alfalfa and sweet clover. Furthermore, the fungus can also attack roots of *Trifolium* spp. It has not been previously reported on any of the legume forage crops, and practically nothing is known concerning its parasitism on other plants. However, Woilenweber (29) reported it as occurring in Europe on decayed roots and other parts of various plants, and it has been found in both cultivated and virgin soils during the present study. Hence, it seems possible that future studies may reveal *C. Ehrenbergi* as an important root parasite of other plants.

Other species of *Cylindrocarpon* are apparently of incidental occurrence as saprophytes or weak parasites on roots of alfalfa and sweet clover, since they have been isolated infrequently, and usually in association with *C. Ehrenbergi*. However, *C. obtusisporum* can cause moderate damage to the roots, and may yet prove of importance. *C. radicicola* has proved weakly parasitic on roots of alfalfa and sweet clover, despite the fact that this species has been reported as a destructive parasite on the roots and bulbs of various other plants. *C. olidum* was the only species studied which showed no evidence of parasitism on the legumes.

The relation of temperature to growth of *C. Ehrenbergi* has proved of special significance in this study. In the first place, the ability of the isolates to grow at a temperature at or slightly below freezing probably explains why they can attack and cause serious damage to roots of alfalfa and sweet clover in the early spring. Also, the isolate that was most pathogenic in the summer, and least virulent in the early spring, grew best at about 24° C., while those causing least injury in summer and maximum damage the following winter belonged to a group having a lower optimum for growth. Hence, these results indicate that certain races of *C. Ehrenbergi* may be chiefly responsible for the damage occurring in the early spring, while other races with a higher temperature relation may attack the roots of growing plants later.

With regard to control measures for *C. Ehrenbergi*, the development of resistant varieties of alfalfa and sweet clover appears to offer the best possibilities. Although the present studies indicate that most of the commonly grown varieties are susceptible to attack by this pathogen, resistant species like *Medicago falcata* may prove valuable as plant breeding material. The possible existence of parasitic races of the fungus is likely to complicate and delay the breeding of resistant varieties. In the meantime, crop rotation is necessary, since *C. Ehrenbergi* increases rapidly in the soil when legume forage crops are grown continuously. Cereal crops are apparently not attacked by the fungus, and it might be advisable to grow them for several years in fields where alfalfa or sweet clover have suffered severely.

Further evidence was obtained in this study that alfalfa and sweet clover are predisposed in the early spring to attack by root-rotting fungi. The critical period for infection usually occurs when the soil is thawing out, and it may last for only a few days. Afterwards, the roots become relatively resistant to invasion by *C. Ehrenbergi*, and, as previously shown by Sanford (23), immune to attack by *Plenodomus Meliloti*. The exact factors concerned in this phenomenon are not yet known. However, since retarded wound-cork formation is apparently not important (9), it seems probable that an altered biological condition of the root tissues, in combination with certain environmental factors, creates the increased susceptibility of the plants as they emerge from the dormant condition.

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References

1. ANLIKER, J. Beiträge zur Kenntnis der Fusariose des Roggens. Beitr. Kryptogamenfl. Schweiz. 8 : 1-115. 1935.
2. ASHCROFT, J. M. European canker of black walnut and other trees. West. Virg. Agr. Expt. Sta. Bull. 261. 1934.
3. BEAUMONT, A. Diseases of narcissi and tulips. Sci. Hort. 3 : 184-191. 1935.
4. BERKELEY, G. H. Root rots of the raspberry. Can. J. Research, C, 14 : 306-317. 1936.
5. BERKELEY, G. H. and LAUDER-THOMSON, I. Root rots of strawberry in Britain. The "black lesion" type of strawberry root rot. J. Pomology Hort. Sci. 12: 222-246. 1934.
6. BISBY, G. R., JAMES, N. and TIMONIN, M. Fungi isolated from Manitoba soil by the plate method. Can. J. Research, 8 : 253-275. 1933.
7. BISBY, G. R., TIMONIN, M. I. and JAMES, N. Fungi isolated from soil profiles in Manitoba. Can. J. Research, C, 13 : 47-65. 1935.
8. CORMACK, M. W. On the invasion of roots of *Medicago* and *Melilotus* by *Sclerotinia* sp. and *Plenodomus meliloti* D. and S. Can. J. Research, 11 : 474-480. 1934.
9. CORMACK, M. W. On wound cork in roots of *Medicago* and *Melilotus*. Proc. World's Grain Exhib. and Conf., Regina, Canada. 1933. 2 : 256-262. 1934.
10. EHRLICH, J. The beech bark disease. A *Nectria* disease of *Fagus*, following *Cryptococcus fagi* (Baer.). Can. J. Research, 10 : 593-692. 1934.
11. FEEKES, F. H. Onderzoekingen over schimmelziekten van bolgewassen. Thesis. Univ. Utrecht. 1931. Abstr. in Rev. Appl. Mycol. 10 : 794-795. 1931.
12. HILDEBRAND, A. A. Root rot of ginseng in Ontario caused by members of the genus Ramularia. Can. J. Research, 12 : 82-114. 1935.
13. HILDEBRAND, A. A. and KOCH, L. W. A microscopical study of infection of the roots of strawberry and tobacco seedlings by micro-organisms of the soil. Can. J. Research, C, 14 : 11-26. 1936.
14. HODGES, F. A. Fungi of sugar beets. Phytopathology, 26 : 550-563. 1936.
15. JENKINS, A. E. *Ramularia macrospora* Fres. (on Rosa). U.S. Dept. Agr. Plant Disease Reporter Supp. 37 : 410. 1925.
16. JOHANSEN, D. A. Dehydration and infiltration. Science, 82 : 253-254. 1935.
17. PELTIER, G. L. and KING, C. J. Studies of *Ozonium* root rot in Arizona and Texas. U.S. Dept. Agr., Dept. Bull. 1417. 1926.
18. REINKING, O. A. *Cylindrocarpon* fungus studies. Zentr. Bakt. Parasitenk. Abt. II, 94 : 134-136. 1936.
19. REINKING, O. A. *Cylindrocarpon* isolations from tropical soils. Zentr. Bakt. Parasitenk. Abt. II. 94 : 137-142. 1936.
20. RICHTER, H. Die wichtigsten holzbewohnenden Nectrien aus fer Gruppe der Krebserreger. Z. Parasitenk. 1 : 25-75. 1928.
21. RIDGWAY, R. Color standards and color nomenclature. Published by the author. Washington, D.C. 1912.
22. ROBBINS, W. J. Isoelectric points for the mycelium of fungi. J. Gen. Physiol. 6 : 259-271. 1924.
23. SANFORD, G. B. A root rot of sweet clover and related crops caused by *Plenodomus meliloti* Dearness and Sanford. Can. J. Research, 8 : 337-348. 1933.
24. SANFORD, G. B. and CORMACK, M. W. On varietal resistance of *Medicago* and *Melilotus* to root rots caused by *Sclerotinia* sp. and *Plenodomus meliloti* D. and S. Proc. World's Grain Exhib. and Conf., Regina, Canada, 1933. 2 : 290-293. 1934.
25. STOUGHTON, R. H. Thionin and Orange G. for the differential staining of bacteria and fungi in plant tissues. Ann. Appl. Biol. 17 : 162-164. 1930.
26. WOLLENWEBER, H. W. *Ramularia, Mycosphaerella, Nectria, Calonectria*. Eine morphologisch pathologische Studie zur Abgrenzung von Pilzgruppen mit cylindrischen und sichelförmigen Konidienformen. Phytopathology, 3 : 197-242. 1913.
27. WOLLENWEBER, H. W. Fusaria autographice delineata. Ann. Mycologici, 15: 1-56. 1917.
28. WOLLENWEBER, H. W. Pyrenomycetenstudien. I. Angew. Botan. 6 : 300-313. 1924.
29. WOLLENWEBER, H. W. Uber Fruchtformen der krebserregenden Nectriaceen. Z. Parasitenk. 1 : 138-173. 1928.
30. WOLLENWEBER, H. W. Fusarium-Monographie. Fungi parasitici et saprophytici. Z. Parasitenk. 3 : 269-516. 1931.
31. WOLLENWEBER, H. W. Aplenveilchen (Zyklamen) Welke, eine Krankheit pilzicher Natur. Nachrbl. deut. Pflanzenschutzdienst, 14 : 38-39. 1935.
32. WOLLENWEBER, H. W. and HOCHAPFEL, H. Beiträge zur Kenntnis parasitärer und saprophytischer Pilze. III. *Fusarium* und *Cylindrocarpon* und ihre Beziehung zur Fruchtfäule. Z. Pflanzenkrank. Pflanzenschutz. 46 : 534-544. 1936.
33. ZELLER, S. M. European canker of pomaceous fruit trees. Oregon Agr. Coll. Expt. Sta. Bull. 222. 1926.
34. ZELLER, S. M. Species of *Nectria, Gibberella, Fusarium, Cylindrocarpon*, and *Ramularia* occurring on the bark of *Pyrus* spp. in Oregon. Phytopathology, 16: 623-627. 1926.

THE EFFECT OF HIGH TEMPERATURE ON UREDIAL DEVELOPMENT IN CEREAL RUSTS[1]

By Thorvaldur Johnson[2] and Margaret Newton[3]

Abstract

The effect of high temperatures on the development of stem rust and leaf rust on wheat seedlings and stem rust and crown rust on oats seedlings was studied in greenhouse experiments. The experimental results show that, for temperatures above the optimum for rust development, the higher the temperature the less vigorous the pustule development. Physiologic races that at ordinary temperatures produce a "4" type of infection tend to develop a "3" type or an "x" type at higher temperatures. At still higher temperatures the infection type becomes "2" or "1" or even merely necrotic flecks. Physiologic races of the same rust differ in their sensitiveness to temperature. In stem rust of wheat, races that had been inbred by repeated selfings for two or more generations, showed greater sensitiveness to temperature than races collected in the field. Leaf rust of wheat and crown rust of oats were less tolerant of high temperatures than stem rust of wheat.

Introduction

It has been shown in recent years by many investigators of the cereal rusts that temperature has a profound influence on the development of the uredial stage of these rusts on their cereal hosts. From results published by Waterhouse (10), Johnson (4), Gordon (3), and Peturson (8), it is clear that a moderately high temperature (about 75° F.) favors maximum pustule development of physiologic races of *Puccinia graminis Tritici* Erikss. and Henn., *Puccinia graminis Avenae* Erikss. and Henn., and *Puccinia coronata Avenae* Erikss. and Henn., whereas temperatures below 60° F. tend to inhibit pustule development in certain physiologic races on some host varieties.

Melander (6) has shown that the development of *Puccinia graminis Tritici* is almost suppressed at a temperature of 0° to 1° C., although the capacity for normal rust development is generally recovered on exposure of the infected plants to higher temperatures.

Recently, evidence has been secured that excessively high temperatures may result in a somewhat similar inhibition of rust development in cereal rusts. During the extremely hot weather that prevailed at Winnipeg in July, 1936, it was observed that seedlings of Little Club wheat developed resistance to certain physiologic forms of *P. graminis Tritici*, to which this variety is normally susceptible. On June 27 and on July 3, seedlings of Little Club were inoculated with four races of wheat stem rust that normally produce a "4" type of infection. During the period June 27 to July 15 the temperature of the greenhouse rose as high as 116° F., with a mean maximum daily temperature of 99.6° F. and a mean minimum daily temperature of 66.8° F. When notes on rust development were taken on July 15, it was observed that the infection types produced by some of the races were distinctly abnormal.

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Thus Race 9 produced an "x−" type of infection, Race 9 (orange) and Race 36 (Sudan brown) produced infection types varying from necrotic flecks to a "2" type of pustule (Fig. 1). Race 48 (Fig. 1), however, seemed to be uninfluenced by the high temperatures. With the advent of cooler weather after July 15, observations were made with the object of determining how far the abnormal infections on these plants were capable of recovering their normal rust development. The "x−" type of infection produced by Race 9 soon developed into an approximately normal "4" type of infection, whereas Race 9 (orange) and Race 36 (Sudan brown) showed no further pustule development.

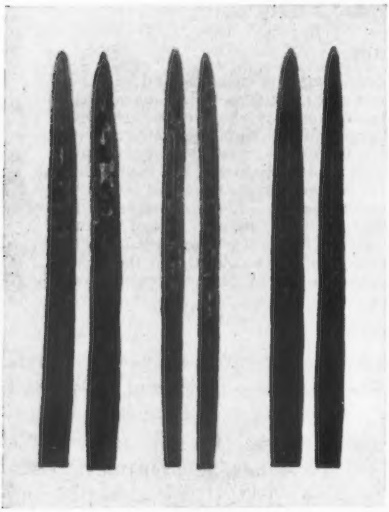

FIG. 1. *The effect of high temperatures (mean maximum 99.6° F., mean minimum 66.8° F.) on the infection types produced by two physiologic races of P. graminis Tritici on Little Club seedlings. Left, Race 48—unaffected by the high temperature. Right, Race 36 (Sudan Brown)—strongly affected by the high temperature. Centre, Race 36 (Sudan Brown)—at approximately normal temperatures.*

These observations led to a series of greenhouse experiments in which the reactions of normally susceptible varieties of wheat and oats to their respective rusts were studied over a wide range of temperature. The rusts selected for this study were *P. graminis Tritici*, *P. graminis Avenae*, *P. coronata Avenae*, and *Puccinia triticina* Erikss. The range of temperature through which these rusts were studied extended from about 55° F. to over 100° F.

Methods

As the main object of this study was to determine the reactions at various temperatures of varieties which are completely susceptible at ordinary greenhouse temperatures, the experiments were for the most part limited to such varieties as Little Club wheat, which is highly susceptible to most physiologic races of stem rust and leaf rust of wheat, and Victory oats which is susceptible to stem rust and crown rust of oats. Seedlings of these varieties were inoculated and kept overnight in damp chambers maintained at a temperature favorable to infection (about 65°–70° F.). On the following morning the pots containing the seedlings were removed from the damp chambers and distributed in equal numbers among compartments of the greenhouse kept at various relatively constant temperatures, in which they remained until notes were taken on rust development. The experiments were conducted during November and December, 1936, and March and April, 1937.

TABLE I

AVERAGE INFECTION TYPES OF PHYSIOLOGIC RACES OF *Puccinia graminis Tritici* ON LITTLE CLUB WHEAT SEEDLINGS AT TEMPERATURES RANGING FROM 55° TO 99° F.

Race	Source	Mean daily temperature								
		55°–59° F.	60°–64° F.	65°–69° F.	70°–74° F.	75°–79° F.	80°–84° F.	85°–89° F.	90°–94° F.	95°–99° F.
19	Field culture	–	–	4	–	–	–	–	–	3 to 4 c.
21	Field culture	–	–	4	–	–	–	–	–	3 to 4 c.
34	Field culture	–	–	4	–	–	–	–	–	3 to 4 c.
49	Field culture	4	4	–	4	–	–	3+ to x	0	–
56	Field culture	–	–	4	–	–	–	–	–	4–
11 Mars yellow	Barberry (F_1)*	4–	4–	4	4–	–	0;	–	0	–
21	Barberry (F_1)	4–	4	–	4	–	–	x+	2+	–
36 Grayish-brown	Barberry (F_3)	–	3+	–	4–	3+	–	x–	0;	0;
36 Ochraceous-buff	Barberry (F_3)	4–	4–	4	4–	–	1–	–	0	0
52 Grayish-brown	Barberry (F_4)	–	4–	4	4	–	–	4–	3 c.	3 c.
57 Orange	Barberry (F_4)	–	4–	–	4–	–	–	0;	0	–

* *F_1, F_3, F_4 are, respectively, first, third, and fourth generation cultures of crosses between physiologic races. The term "generation", as here used, represents the passage of the rust through its complete life cycle: uredia→telia→aecia→uredia.*

c. = *sharply chlorotic areas surrounding pustules.*

Experiments with Physiologic Races of *Puccinia graminis Tritici*

In view of the fact that the different physiologic races on which observations were made in July, 1936, did not show an identical response to the high temperatures prevailing at that time, it was decided to include in subsequent experiments several physiologic races from various sources. Accordingly these tests included not only races isolated from field collections, but also several races originating in crossing and selfing studies carried out at the Dominion Rust Research Laboratory. In Tables I and II, races from the latter source have been designated by the term "Barberry" to distinguish them from races collected in the field.

Table I shows the infection types produced on Little Club wheat by 11 cultures of wheat stem rust at various temperatures between 55° F. and 99° F. The symbols used to record the types of infection are those originally described by Stakman and Levine (9). The infection types recorded in the table are, in most cases, averages secured from several tests.

An examination of Table I makes it clear that physiologic races differ considerably in their response to high temperatures. It is apparent also that the races collected in the field are less affected by high temperature than those derived from crosses or repeated selfings. The behavior of the field collection of Race 49 at temperatures above 85° F., however, shows that races collected in the field are not all equally resistant to high temperatures. Similarly, there are considerable differences in the temperature responses of the cultures derived from the barberry. Race 52 (grayish-brown), as shown in Table I, and Races 36 and 15 (white), in Table II, exhibit a somewhat greater toleration of high temperatures than other barberry cultures.

TABLE II

THE EFFECT OF TEMPERATURE ON INFECTION TYPE AND NUMBER OF PUSTULES PRODUCED BY FOUR PHYSIOLOGIC RACES OF *P. graminis Tritici* ON LITTLE CLUB SEEDLINGS

Race	Source	Mean daily temperature					
		60.4° F.		78.7° F.		89.7° F.	
		Infection type	Number pustules	Infection type	Number pustules	Infection type	Number pustules
36	Field culture	3+	53	4	10	4	10
36	Barberry (F_2)	3+	98	4	36	4 c.	6
36 Grayish-brown	Barberry (F_3)	3	79	3+c.	4	x	3
15 White	Barberry (F_4)	3	175	4 c.	33	4 c.	11

Throughout all the experiments, it was noted that the higher the temperature at which the host plants were kept, the fewer pustules developed on the plants. Characteristic results are shown in Table II which records an experiment in which a count was made of the number of pustules formed on the same number of seedlings at three different temperatures. As the infections took place under identical conditions (in this and all other experiments) the sparseness of pustule development at the higher temperatures is clearly the result of a suppression, in an early stage, of mycelial growth in many infections at the higher temperatures.

Experiments with Physiologic Races of *Puccinia triticina*

The results of a number of tests conducted with physiologic races of *Puccinia triticina* are summarized in Table III. The effect of temperature is clearly manifested at temperatures above 85° F. in a tendency on the part of the host plant to develop resistance. It would appear that Race 35 is more sensitive to high temperatures than the other races tested.

TABLE III

AVERAGE INFECTION TYPES OF PHYSIOLOGIC RACES OF *Puccinia triticina* ON LITTLE CLUB WHEAT SEEDLINGS AT TEMPERATURES RANGING FROM 55° TO 94° F.

Race	Mean daily temperature						
	55°–59° F.	60°–64° F.	65°–69° F.	70°–74° F.	75°–79° F.	85°–89° F.	90°–94° F.
5	3+	3+	–	3+	3	x	0;
35	–	4–	–	4–	–	1±	–
76	–	3+	–	–	3	x	–
80	–	3+	3+	4–	–	x–	0; to x–

Experiments with Physiologic Races of *Puccinia coronata Avenae* and *Puccinia graminis Avenae*

The results of these experiments are summarized in Table IV. Seedlings of the variety Victory were used as experimental plants, as this variety is susceptible to races of both rusts. These rusts appeared to be even more

TABLE IV

AVERAGE INFECTION TYPES OF PHYSIOLOGIC RACES OF *Puccinia coronata Avenae* AND *Puccinia graminis Avenae* ON VICTORY OATS SEEDLINGS AT TEMPERATURES RANGING FROM 60° TO 94° F.

Race	Mean daily temperature					
	60°–64° F.	70°–74° F.	75°–79° F.	80°–84° F.	85°–89° F.	90°–94° F.
1 *P. coronata Avenae*	3+	–	–	–	0 cn.	–
3 *P. coronata Avenae*	3+	3+	3 c.	3±cn.	3 cn.	–
24 *P. coronata Avenae*	3+	3+	3+c.	–	3–cn.	1 cn.
6 *P. graminis Avenae*	4–	4	–	–	–	0 cn.

c. = *chlorotic spots.* cn. = *chlorotic and necrotic spots.*

sensitive to temperature than the wheat rusts. At a temperature slightly above 75° F., Races 3 and 24 of *P. coronata Avenae* showed a visible reaction to temperature in the development of sharply chlorotic areas around the pustules. At higher temperatures the chlorosis was largely replaced by necrosis. At temperatures above 85° F. pustules were few, when present at all, but chlorotic spots and necrotic lesions of a brown color were numerous. That the absence of pustules was not due to failure of germ-tube penetration

was demonstrated in leaves of Victory that had been inoculated by *P. coronata Avenae* and kept for 10 days at a mean temperature of 90° F. These leaves, when stained according to McBryde's method (5) of demonstrating rust hyphae in unsectioned leaf tissue, showed here and there mycelia of limited development (Fig. 2). It is not altogether clear what relation, if any, the brown, necrotic areas have to infections. They appeared occasionally on uninoculated, control seedlings at high temperatures but in much smaller numbers than on the inoculated plants. Although no mycelia could be seen in the necrotic lesions when they were stained according to the above-mentioned method, it is possible that they were present but could not be detected owing to the tendency of the dead plant tissue to stain the same color as the mycelium.

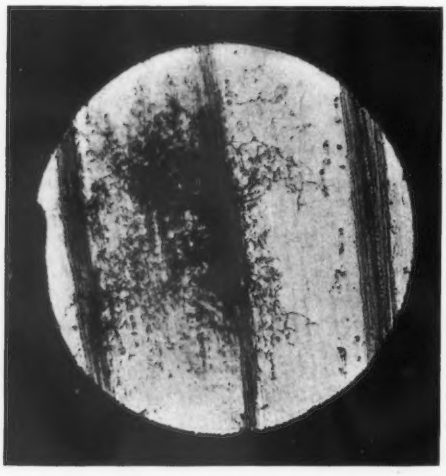

FIG. 2. *Mycelium of P. coronata Avenae, Race 24, in a 10-days old infection on a seedling of Victory oats kept at a mean temperature of 90° F.* ×50.

The limited experiments on oat stem rust do not permit any definite conclusions to be drawn but suggest that its behavior towards temperature is similar to that of crown rust.

Discussion

A consideration of the experiments reported in this paper makes it clear that there is an optimum range of temperature for the development of cereal rusts and that progressively higher temperatures militate against normal rust development. Melander (6) has shown that temperatures much lower than the optimum produce a somewhat similar effect. It would appear that the host plants—both wheat and oats—can tolerate a wider range of temperature than their respective rusts. No attempt was made, in the experiments reported above, to determine the absolute upper limit of temperature which wheat or oats will tolerate; and it is, indeed, probable that varieties differ somewhat in this respect. At the highest temperatures tested (mean temp. 97° F.), Little Club wheat did not appear to suffer appreciable injury, providing that soil moisture conditions were satisfactory, whereas even the most vigorous cultures of stem rust tested produced few pustules and these were rather small and surrounded by sharp chlorosis. Leaf rust is apparently less capable of withstanding high temperatures than stem rust; at a mean temperature of 94° F. it failed, for the most part, to produce pustules, and

in one experiment Little Club was rendered highly resistant at a mean temperature of 86° F. Crown rust of oats appears to possess about the same degree of toleration towards temperature as leaf rust of wheat. Experiments on the reaction of stem rust of oats to temperature were too few to permit any definite conclusions.

In stem rust of wheat, and to a lesser extent in leaf rust and crown rust of oats, considerable differences were noted in the sensitiveness of different physiologic races to high temperature. In stem rust of wheat the cultures derived from the barberry were definitely more sensitive to high temperature than those originating in field collections (*Vide* Table I). It must not be inferred from this observation that all cultures derived from aecia on the barberry will behave in a similar way. Most of the "barberry" cultures studied were F_3 or F_4 cultures derived from crosses made several years ago at the Dominion Rust Research Laboratory. The repeated selfings to which such cultures are subjected frequently bring to light various abnormal characteristics such as abnormalities of spore color or a decrease in vigor of sporulation. The appearance of such characteristics is probably due to homozygosity of the factors governing them, a condition brought about by repeated selfings. These factors were undoubtedly present in a heterozygous state in the original rust or F_1 hybrid but, being recessive, they would produce no visible effects. It seems probable that the sensitiveness of the "barberry" cultures to a high temperature is merely one form of degeneration consequent on the continued selfing of physiologic races.

From the point of view of host reaction, the effect of high temperature is expressed in progressively increasing resistance at progressively higher temperatures. The first indication of resistance is the formation of sharply defined chlorotic areas surrounding the pustules. At a higher temperature the infection type ceases to be a "4" or a "3" and becomes an "x" type, that is, pustules of a resistant and susceptible type are intermingled. At still higher temperatures the infection types become "2" or "1" or even merely necrotic flecks. Thus a variety, susceptible at moderate greenhouse temperatures, may exhibit various degrees of resistance at higher temperatures.

The question of whether the host plant is able to maintain a resistance thus acquired, if transferred to a lower temperature, was not thoroughly investigated. A few experiments were performed in an attempt to gain some information on this point. Seedlings of Little Club, which had acquired resistance to stem rust at a high temperature, were subsequently kept at a moderate temperature to determine to what extent the usual infection type of the rust was recovered. The results were not entirely consistent. On certain leaves, which bore only necrotic or chlorotic flecks at the high temperature, pustules of a "3" or "4" type would later develop at the lower temperature. On other leaves, the resistance acquired at the high temperature would be retained at the lower. The mycelium, therefore, had survived in some infections but not in others. A sufficiently long exposure of the mycelium to high temperatures would probably lead to its destruction in all of the infections.

It is probable that the response of these rusts to high temperature has some significance in their epidemiology. That such is the case for *Puccinia glumarum* has been established by Gassner and Straib (1, 2) who have used the term "Sommerresistenz" to designate the resistance which many varieties develop towards stripe rust in the summer months. The sensitiveness of *P. glumarum* to high temperature has also been invoked by Newton and Johnson (7), to account for the failure of stripe rust to spread in the prairie provinces of Western Canada during the midsummer period. It is possible that the relatively smaller damage done by the leaf rust of wheat and crown rust of oats in the great plains region than by stem rust is to some extent attributable to a similar cause. The behavior of leaf rust of wheat particularly is suggestive. In Manitoba this rust usually appears early in the summer and spreads considerably while the weather is still cool. With the advent of warmer weather, leaf rust makes much slower progress than stem rust although the latter appears somewhat later in the season. Possibly the greater resistance of stem rust to high temperature may, at least in part, account for its rapid development in periods of warm weather.

References

1. GASSNER, G. und STRAIB, W. Experimentelle Untersuchungen über das Verhalten der Weizensorten gegen *Puccinia glumarum*. Phytopath. Z. 1 : 215-275. 1929.
2. GASSNER G. und STRAIB, W. Experimentelle Untersuchungen zur Epidemiologie des Gelbrostes (*Puccinia glumarum* [Schm.] Erikss. und Henn.). Phytopath. Z. 7 : 285-302. 1934.
3. GODRON, W. L. Effect of temperature on host reactions to physiologic forms of *Puccinia graminis avenae* Erikss. and Henn. Sci. Agr. 11 : 95-103. 1930.
4. JOHNSON, THORVALDUR. A study of the effect of environmental factors on the variability of physiologic forms of *Puccinia graminis tritici* Erikss. and Henn. Dom. Dept. Agr. Bull. No. 140. N.S. 1931.
5. MCBRYDE, MARY C. A method of demonstrating rust hyphae and haustoria in unsectioned leaf tissue. Am. J. Botany 23 : 686-688. 1936.
6. MELANDER, LEONARD W. Effect of temperature and light on the development of the uredial stage of *Puccinia graminis*. J. Agr. Research, 50 : 861-880. 1935.
7. NEWTON, MARGARET and JOHNSON, T. Stripe rust, *Puccinia glumarum*, in Canada. Can. J. Research, C, 14 : 89-108. 1936.
8. PETURSON, B. Effect of temperature on host reactions to physiologic forms of *Puccinia coronata avenae*. Sci. Agr. 11 : 104-110. 1930.
9. STAKMAN, E. C. and LEVINE, M. N. The determination of biologic forms of *Puccinia graminis* on *Triticum* spp. Minn. Agr. Exp. Sta. Tech. Bull. 8. 1922.
10. WATERHOUSE, W. L. Australian rust studies I. Proc. Linnean Soc. N.S.W. 54 : 615-680. 1929.

STIMULATION OF CAMBIAL ACTIVITY, LOCALLY IN THE REGION OF APPLICATION AND AT A DISTANCE IN RELATION TO A WOUND, BY MEANS OF HETEROAUXIN[1]

By A. B. Brown[2] and R. G. H. Cormack[3]

Abstract

The application of heteroauxin in lanoline (1 mg. of heteroauxin per gm. of lanoline) to the distal end of disbudded cuttings of leader shoots of balsam poplar, stimulated cambial activity for a distance of 1.0–1.5 in. below the point of application. Marked stimulation of local cambial activity, in relation to a bridged ring some considerable distance below the point of application of the heteroauxin, was also obtained. The response at the wound was distinct and separate from the response in the region of application of the heteroauxin, since in the intervening distance no cambial activity had occurred. The experiments were carried out during the winter months, so that the cambium was dormant in material as it came from the field. Cambial activity subsequent to treatment was estimated in terms of xylem formation. The structural features of this new xylem are described and discussed, with particular reference to the question as to whether heteroauxin stimulates cell division only in the cambium or, in addition, is active in differentiation of typical xylem elements.

Introduction

In a recent contribution Brown (2), using leader shoots of balsam poplar, showed that in disbudded cuttings the greater the amount of living bark distal to a bridged wound, the greater is the development of local cambial activity in relation to the wound. It was also shown that local wound-cambial activity is stimulated further by the presence of developing buds and leaves distal to the wound. This work was done during the winter months, at a time when the cambium was dormant in material outdoors, which rendered it easy to measure cambial activity subsequent to treatment. On the basis of the quantitative results obtained, it was concluded that a hormone, present in the living bark and produced also by developing extension growth, is involved in local wound-cambial activity. Moreover, it was argued further that this hormone is probably identical with that which emanates from developing extension growth to promote the basipetal development of normal cambial activity.

The concept of hormone regulation of normal cambial activity receives strong support from the fundamental investigations of Avery, Burkholder and Creighton (1), and of Söding (6), who have definitely established a close parallelism between the intensity of cambial activity and growth-hormone concentration. In their experiments, the concentration of growth hormone was estimated in terms of the *Avena* coleoptile test, and it is implied that the hormone promoting cell extension or elongation promotes cell division in the cambium also. Such a conclusion had been reached at an earlier date by Snow (4), who suggested that the cambial hormone and the growth hormone were identical, and might indeed be auxin-*a*.

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Snow (4) succeeded in activating the cambium in decapitated stems and hypocotyls of sunflower seedlings by applying to their upper ends weak solutions in gelatine of pure heteroauxin (3-indole acetic acid) and of auxin-*a*. Söding (5) obtained activation of the cambium in shoots of various trees as a result of application of pure heteroauxin. The twigs were ringed towards the end of March, and buds below the ring were removed. About two months later, he made a longitudinal incision running down 2–3 cm. from the lower margin of the ring, a flap of bark was lifted up and crystalline heteroauxin applied directly to the exposed cambium. After periods of 16–21 days and one month, in the experiments of Snow and Söding respectively, stimulation of the cambium was observed for a few centimetres below the point of application of the auxin. According to Söding (6) similar results with heteroauxin have recently been obtained by at least two other European investigators.

Now although heteroauxin, unlike auxins *a* and *b*, has never been isolated from the higher plants, experiments with this substance upon higher plants are of interest, because in all the phenomena which have so far been investigated, it has acted in the same way as the other auxins. With regard to wound-cambial activity, it is clearly of interest to determine whether it is possible to stimulate cambial activity in the vicinity of a wound by the application of heteroauxin at a point some distance above the wound, and in such a way that any response at the wound is distinct from any development arising in the region of application of the auxin. Such a result would be analogous to that obtained (2) when developing buds and leaves are present distally on a cutting, since stimulation of local cambial activity in relation to a wound below can be observed definitely before the basipetal gradient of normal cambial activity, emanating from the developing extension growth, has reached the level of the wound.

The difficulty in explaining local cambial activity in relation to bridged wounds, wholly in terms of the action of a single hormone, has already been discussed by Brown (2), who has suggested a hypothesis involving interaction between the hormone and a definite wound substance. This however is another problem and the original paper should be consulted for details.

Experiments and Results

Leader shoots of *Populus balsamifera* L., the balsam poplar, were used throughout this investigation. In the first experiment to be described, the three-year-old portions of six leader shoots were selected (December 29, 1936), and these lengths of shoot were completely disbudded by the excision of all lateral branches. Each length of shoot was then cut to form a set of three units of equal length, which will be designated as the upper, middle and lower units in each set. Although the three units in any one set were all of equal length, the different sets varied in length from 12 to 15 inches.

In each set a similar longitudinally bridged ring was made at the same distance from the morphologically upper end in all three units. The distance

from the upper end of the unit to the bridged ring below varied from 7–10 in. in different sets. Likewise the length and width of the longitudinal bridge of bark varied somewhat in different sets, the average length of the bridge being about $\frac{3}{4}$ in., and the width approximately $\frac{1}{12}$ of the circumference of the unit. The distance between the bridged wound and the basal end of the unit was from 4 to 5 inches.

Variation between different sets is of no import in so far as this work is concerned, and is indeed difficult to avoid in many cases, simply because of the constitution of the material with respect to variation in thickness of the shoots, in the length of internodes, and so on. What is important is to ensure, as far as is possible, uniformity between units within a set, and this was carefully attended to in these experiments. The bridged wound was smeared with vaseline and the units set up in a vertical position with their basal end in about one inch of tap water. It is necessary to keep all the units vertical since, as Brown (2) has shown, gravity has a marked influence upon cambial activity in relation to wounds.

About two weeks later (January 13, 1937) some of the bark was scraped off all around for a short distance ($\frac{1}{2}$ in.) from the upper end of all units, to expose the living phloem beneath. In two units of each set, namely the upper and lower, the exposed tissue was smeared liberally with a lanoline paste (1 part anhydrous woolfat to 1 part distilled water) containing 1 mg. of pure heteroauxin per gm. of lanoline. The heteroauxin (3-indole acetic acid) was a sample purchased from Merck and Co. The middle unit served as a control and was treated similarly, except for the fact that the lanoline paste contained no heteroauxin. On January 29, and again on February 3, the respective lanoline pastes were removed and renewed, and on each occasion the previously exposed phloem was scraped again to present a fresh surface. The experiment terminated four weeks after the first of the three treatments with heteroauxin (February 2, 1937), when the bark was peeled off entirely and the units allowed to dry out.

On drying out, any new xylem laid down by the cambium during the experiment shows up clearly upon the surface of the old wood. Fig. 1 is a photograph of a typical set showing the response at the bridged wound, and at the distal end of the upper (*a*), middle (*b*) and lower (*c*) units. The material illustrated was first photographed without any previous treatment, except for the fact that the new xylem was outlined with India ink. However, it became clearly evident that there was insufficient differentiation in the photograph to allow for reproduction, and accordingly the following procedure was resorted to. Powdered brown chalk was rubbed lightly into the wood with the finger. The chalk adhered readily to the rough surface of the new xylem but not to the smooth surface of the old, and in this way marked differentiation was obtained. It will be observed in the upper and lower units, which were treated with heteroauxin, that a basipetal gradient of new xylem has been laid down for a short distance (1.0–1.5 in.) from the distal end of the units, whereas in the middle control unit no such response is evident.

But of still greater interest is the fact that there is obviously much more new xylem in the vicinity of the bridged wound below in the treated units *a* and *c*, relative to that in the control unit *b*. Moreover, the response at the bridged wound in the treated units is distinct and separate from the response at the distal end, since no new xylem had been laid down in the intervening distance, except locally in some cases at the base of excised lateral shoots. Transverse sections were made through the middle of the longitudinal bridge in all units, and the number of vessels counted. Table I shows the results obtained for the six sets. The first set in Table I is that which had been photographed to provide Fig. 1. These vessel counts indicate clearly that the application of a heteroauxin paste some distance above has greatly stimulated the cambium in the vicinity of a bridged ring below. In every case, the response at the bridged ring was distinct and separate from the response in the region of application of the heteroauxin.

TABLE I

NUMBER OF VESSELS IN A TRANSVERSE SECTION THROUGH THE LONGITUDINAL BRIDGE OF UNITS TREATED WITH HETEROAUXIN AND OF CONTROL UNITS

Upper (treated)	Middle (control)	Lower (treated)
330	80	290
286	53	333
351	84	344
330	33	460
167	15	201
106	17	133

Similar results were obtained in another experiment. In this case the four-year-old portions of twelve leader shoots were selected (December 29, 1936). These portions were completely disbudded and each portion then cut to form a set of two units of equal length, which will be designated as the upper and lower units of each set. In each set a longitudinally bridged ring was made in both units at the same distance from the morphologically upper end. The average length of the units was 15 in., the average distance between the morphologically upper end and the bridged wound below, 9 in., the average length of the longitudinal bridge of bark $\frac{3}{4}$ in., the average width of the bridge $\frac{1}{12}$ of the circumference of the unit, and the average length of shoot below the bridged ring was $5\frac{1}{4}$ in. Just as before, these lengths varied between sets but were the same for the two units within any one set. The bridged wound was vaselined and the units set up vertically with their basal end in tap water.

The material was treated immediately (December 29, 1936) with heteroauxin. Some of the bark was scraped off all around at the upper end for a distance of about $\frac{1}{2}$ in. to expose the living phloem beneath. In six sets a lanoline paste, containing one mg. of heteroauxin per gm. of lanoline, was applied liberally to the exposed phloem at the end of the lower units. The six upper units served as controls and were treated with lanoline only. In the other six sets the upper units were treated with heteroauxin and the lower units served as controls. The respective pastes were removed and renewed at weekly intervals, at which times the phloem was scraped again to expose a fresh surface. The experiment terminated (February 3, 1937) five weeks after the first of five treatments. As in the previous experiment, the bark was peeled off completely and the material allowed to dry out.

The results were precisely the same as before. In the units treated with heteroauxin, a basipetal gradient of new xylem had been laid down for a distance of 1.0–1.5 in. from the upper end, whereas no such response was obtained in the controls. There was a marked increase in the amount of new xylem in the vicinity of the bridged wound in treated units relative to the controls, and again there was no evidence of cambial activity in the region between the bridged ring and the basipetal gradient of new xylem just below the point of application of the heteroauxin. A quantitative estimate of the difference between the extent of cambial activity at the bridged ring, in treated and control units, is presented in Table II, in which is set forth the number of vessels in transverse sections through the middle of the longitudinal bridge of all units.

TABLE II

NUMBER OF VESSELS IN A TRANSVERSE SECTION THROUGH THE LONGITUDINAL BRIDGE OF UNITS TREATED WITH HETEROAUXIN AND OF CONTROL UNITS

Upper (control)	Lower (treated)
95	335
56	306
57	381
110	405
60	343
76	282
Upper (treated)	**Lower (control)**
503	136
313	114
392	77
118	54
243	85
259	78

Examination of the new xylem, laid down in a basipetal gradient for a short distance just below the region of application of the heteroauxin, showed it to be abnormal in some respects. The wood was characterized by abundant nests of parenchyma, and the vessels were rather narrow on the whole. These abnormalities were most marked at the extreme end of the unit, and the wood became more and more typical at increasing distances from the cut end. Exactly similar observations have been reported by Söding (5) for balsam poplar and a number of other trees. Fig. 2 is a photograph of a transverse section cut at about $\frac{1}{2}$ in. from the distal end of a unit treated with heteroauxin. The section was stained with phloroglucin, a lignin stain, and shows clearly the unstained nests of parenchyma in the new xylem.

Now although there was no obvious response below the distal end of control units, it would not be correct to say that the cambium had remained entirely inactive. Brown (2) has already shown that a certain amount of cambial activity does develop from the lower margin of a complete ring, or what is really the same thing, from the distal cut end of a cutting. Under such conditions a basipetal gradient of cambial activity, very much feebler and less extensive than that obtained when heteroauxin is present, can be observed. The cambium cuts off cells which remain more or less uniformly rectangular in shape, and thin-walled. Vessels and fibres are not differentiated, although a few tracheids may be formed. For a fuller discussion and illustrations of this type of development, the earlier paper (2) should be consulted. It is quite clear, however, from these present experiments, that the effect of heteroauxin is not simply to induce some degree of differentiation in layers of cells which would have been formed, as in the controls, in the absence of hetero-

auxin. Heteroauxin definitely stimulates cell division for a short distance below the point of application.

When the new wood laid down locally in relation to the bridged wound was examined in treated and control units, the following differences, apart from the obvious difference in amount, were noted. Lignification of the new xylem was more marked and there was a tendency for the vessels to be wider in the units treated with heteroauxin than in the controls. It is of interest that similar differences have already been observed by Brown (2) between units bearing developing extension growth, and completely disbudded units. In addition, nests of parenchyma were less common in the new wood formed locally, in relation to the bridged ring, in units treated with heteroauxin than in the controls.

In every unit treated with heteroauxin there was less parenchyma in the wood laid down in the longitudinal bridge of the ring below, than in the basipetal gradient of new xylem in the region of application of the heteroauxin. The wood in the longitudinal bridge in treated units was, generally speaking, quite normal in appearance. Lignification was more marked, and invariably the vessels in this region were wider than the vessels in the basipetal gradient at the distal end of the same unit. Some of these differences are illustrated in Figs. 2, 3 and 4. The material all belonged to the set appearing first in Table II. Fig. 2 is a transverse section about ½ in. below the distal end of the unit treated with heteroauxin; Fig. 3 is a transverse section through the middle of the longitudinal bridge of the same unit; and Fig. 4 is a transverse section through the middle of the longitudinal bridge of the control unit. Only small portions of the bridge are shown in Figs. 3 and 4, but they are from corresponding positions in both units. It will be observed that the wood in the longitudinal bridge of the control unit rather resembles that formed just below the region of application of the heteroauxin in the treated unit. All the sections were stained with phloroglucin in the presence of hydrochloric acid.

The question of tissue orientations in relation to bridged wounds has already been discussed by Brown (2), and nothing would be gained by reconsidering it at this time. Suffice it to say that the tissue orientations round the longitudinally bridged wound were exactly the same in units treated with heteroauxin and in controls.

In all units, a basifugal gradient of xylem was laid down over a short distance at the basal end. This type of development is well known, having been first observed by Hartig (3) in 1862, and commented upon by several investigators since that time. In the present experiments, this basifugal gradient was always more extensive in units treated above with heteroauxin than in the control units, which is just what might be expected. The interesting point, however, is that the new xylem was always markedly "piled up" in the control units, causing a definite bulge just above the cut end, whereas in the treated units the same degree of "piling up" was never evident. In other words, the basifugal gradient of new xylem in the controls was short and steep, whereas

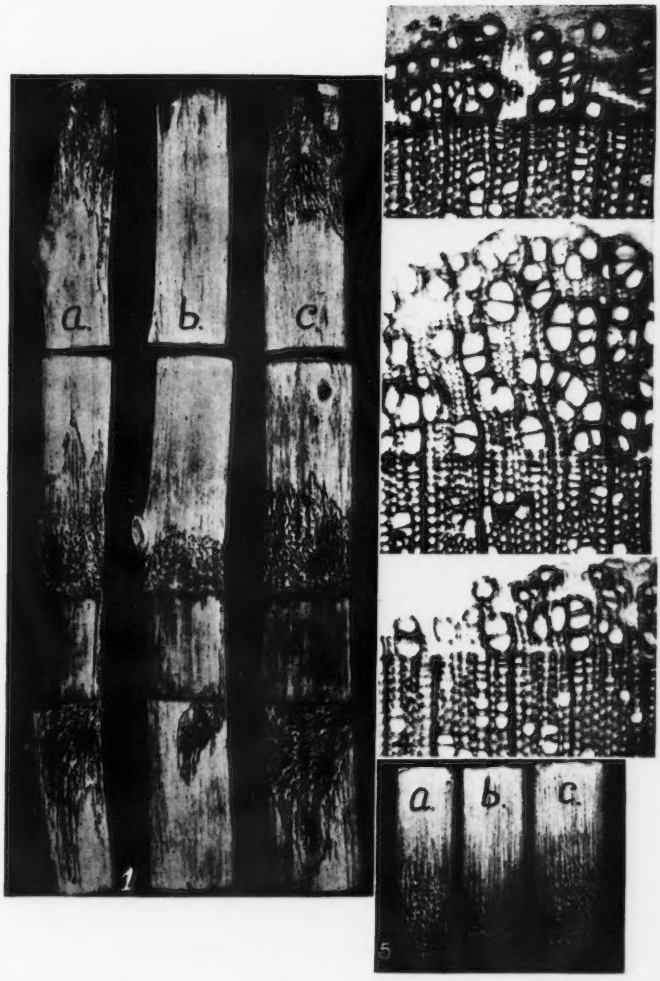

FIG. 1. *Cambial activity at the distal end and around a longitudinally bridged ring below, in three units derived from the three-year-old portion of one leader shoot of balsam poplar. The upper (a) and lower (c) units were treated at the distal end with heteroauxin in lanoline. The middle unit (b) served as a control and was treated with lanoline only.* $\frac{2}{3}$ *nat. size.*

FIG. 2. *Transverse section, cut* $\frac{1}{2}$*-inch from the distal end of a unit treated with heteroauxin.* ×85.

FIG. 3. *Transverse section through the middle of the longitudinal bridge of the same unit as in Fig. 2.* ×85.

FIG. 4. *Transverse section through the middle of the longitudinal bridge of the control unit corresponding to that in Figs. 2 and 3. The sections in Figs. 2, 3 and 4 were stained with phloroglucin and hydrochloric acid. Only a small portion of the longitudinal bridge is shown in Figs. 3 and 4, but from corresponding positions in both cases.* ×85.

FIG. 5. *Cambial activity at the basal ends of a set of three units, derived from the three-year-old portion of one leader shoot. The upper (a) and lower (c) units were treated at the distal end with heteroauxin. and the middle unit (b) served as a control.* $\frac{2}{3}$ *nat. size.*

in the treated units the gradient, although much longer, was much less steep relative to the controls. Indeed, it was quite common to find a greater thickness of new xylem in the region of "piling up" in the control units, than at any point in the basifugal gradient of the corresponding units treated with heteroauxin. Some of these points are illustrated in Fig. 5, which is a photograph of the basal end of the upper (*a*), the middle (*b*) and the lower (*c*) units of the set appearing second in Table I. The material was treated with powdered chalk as previously described.

These observations with respect to the basifugal gradient of xylem at the basal end of units are incidental, to the extent that they do not effect the main conclusions to be derived from these experiments. They may, however, prove to be of significance in other connections, and since they were a constant feature of the experiments, the following tentative explanation is submitted for consideration. If, in the control units, there is a mass movement downwards of growth hormone present in the living bark, there would be a tendency for this hormone to accumulate at the basal end to give rise to a concentration gradient, highest at the base and decreasing in the upward direction. It is also probable that polarity within the unit would tend to steepen this gradient, whereby it is conceivable that the concentration of growth hormone would become, and would be maintained, sufficiently high to stimulate cambial activity, only over a very short distance at the basal end. In which case, the growth hormone would be effective within a very short distance, thus accounting for the "piling up" effect in control units. In the units treated with heteroauxin, there would again be a tendency to form a similar concentration gradient of growth hormone at the basal end as a result of mass movement downwards. In addition, however, there is a concentration gradient of heteroauxin in the opposite direction; *i.e.*, decreasing from the source above downwards. It is clear that on reaching the basal end, the effect of the heteroauxin would be to lengthen the effective concentration gradient of cambial stimulant (in this case growth hormone plus heteroauxin), and in addition to decrease the steepness of that gradient. In this case, the concentration of heteroauxin and growth hormone at the basal end would become sufficiently high to stimulate cambial activity over a considerably greater distance, and without the same degree of "piling up" relative to that obtaining in the controls.

Discussion

The foregoing experiments confirm the earlier conclusions of Snow (4), Söding (5) and others, to the effect that heteroauxin stimulates cambial activity in the shoots of plants for a short distance below the point of its application. Heteroauxin applied at the distal end of a shoot cutting will also stimulate cambial activity around a bridged wound below. Of particular interest is the fact that the response at the wound below is distinct and separate from the response in the region of application of the heteroauxin. The simplest explanation is that the heteroauxin travels down a considerable length of shoot without stimulating the cambium in its path, but does,

however, on reaching a bridged ring below, stimulate markedly local cambial activity at that point. It is quite probable that the bridged wound presents an obstacle to the downward movement of the heteroauxin which will, in consequence, tend to accumulate just above the wound. There is a clear analogy here with the type of behavior obtained when developing extension growth is present distally on a cutting. Brown (2) has shown that local cambial activity in relation to a bridged ring is stimulated by the presence of developing buds and leaves distal to the ring, and that this response can be observed before the normal basipetal development of cambial activity, emanating from the extension growth, has reached the wound. In this connection it was suggested that the hormone emanating from the extension growth must move to some extent in advance of the basipetal development of cambial activity. This suggestion has since been proved correct by Avery, Burkholder and Creighton (1), who have shown definitely that growth hormone moves basipetally in stems in advance of cell division in the cambium. Likewise, Söding (6) found by analysis of plant parts that growth hormone appears first and is followed later by cambial activity.

Söding (5, 6) has recently developed the hypothesis that cambial activity can of itself produce growth hormone. He considers the production of growth hormone in expanding buds to be prerequisite to the initiation of cambial activity in the shoot immediately below, where much of the growth hormone is used up. He then suggests that the activated cambium produces further supplies of growth hormone, which travel but a short distance downwards to repeat the process. In terms of this hypothesis, according to Söding, a molecule of growth hormone some distance down a tree has not travelled to that point from far above, but was manufactured, either in the place in which it finds itself or but slightly above. However, Brown (2) has attributed the stimulation of local wound-cambial activity, when developing extension growth is present on a cutting, to movement of growth hormone in advance of the basipetal development of normal cambial activity. It is admitted that the distance between the advancing front of the basipetal development of cambial activity emanating from the extension growth and the bridged wound below, was never more than a very few inches at the time the degree of stimulation at the bridged ring was estimated. Movement of growth hormone over the space of a few inches might well fall within the terms of Söding's hypothesis. On the other hand, it is quite probable that stimulation of cambial activity at the bridged wound could have been detected earlier, although less clearly of course, at times when greater distances intervened between the wound and the advancing front of cambial activity from above. Under these circumstances, movement of growth hormone over correspondingly greater distances would have to be admitted. The foregoing experiments with heteroauxin are, by analogy, of considerable interest in this connection. Distances as great as nine inches intervened between the lower limits of the basipetal development of cambial activity in the region of application of the heteroauxin and the bridged wound below, where marked stimula-

tion of cambial activity was observed. Still greater distances intervened between the region of application of the heteroauxin and the basal end of cuttings, where the effect of the heteroauxin was also manifested. It would appear reasonable to suppose that the heteroauxin had travelled over these distances, and there is no reason to believe that still greater distances could not be traversed.

Söding (5, 6) has expressed the opinion that growth hormone stimulates cell division only in the cambium, and that other factors are necessary for the differentiation of typical xylem and phloem. This opinion appears to be based mainly on the results of his experiments with heteroauxin, where he found that the new xylem formed just below the point of application of the heteroauxin was abnormal. Differentiation was incomplete in so far as an abundance of parenchyma was present, and to the extent that the vessels were often quite narrow. Similar results were obtained in the present experiments with respect to the xylem formed in the region of application of the heteroauxin, although as Söding (5) also observed, the xylem became more typical at increasing distances below the point of application of the heteroauxin. Söding (6) has refuted the suggestion that an inhibiting effect, due to high concentrations of heteroauxin at the point of application, is the sole cause of incomplete differentiation, since he obtained similar results with weak concentrations. He adheres to his previous suggestion that heteroauxin stimulates cell division only and that other factors are necessary for the differentiation of typical xylem and phloem. However, in view of the results of the present investigation, it might be argued that heteroauxin does indeed stimulate both cell division and differentiation, as is apparently the case in the longitudinal bridge of treated units, and that some other factor limits differentiation at the distal end. It should be recalled that the degree of differentiation at the distal end increases at increasing distances from the region of application of the heteroauxin, which would indicate the action of some purely local factor at more distal points.

References

1. Avery, G. S., Burkholder, P. R. and Creighton, H. B. Production and distribution of growth hormone in shoots of *Aesculus* and *Malus* and its probable role in stimulating cambial activity. Am. J. Botany, 24 : 51-58. 1937.
2. Brown, A. B. Activity of the vascular cambium in relation to wounding in the balsam poplar, *Populus balsamifera* L. Can. J. Research, C, 15 : 7-31. 1937.
3. Hartig, Th. Über die Bewegung des Saftes in den Holzpflanzen. Botan. Ztg. 20 : 70-76, 81-87, 97-100, 105-109. 1862.
4. Snow, R. Activation of cambial growth by pure hormones. New Phytologist, 34 : 347-360. 1935.
5. Söding, H. Über den Einfluss von Wuchsstoff auf das Dickenwachstum der Bäume. Ber. deut. botan. Ges. 54 : 291-304. 1936.
6. Söding, H. Wuchsstoff und Kambiumtätigkeit der Bäume. Jahrb. wiss. Botan. 84 : 639-670. 1937.

CHEMICAL WEED KILLERS

III. RELATIVE TOXICITY OF SEVERAL CHEMICALS TO PERENNIALS UNDER FIELD CONDITIONS[1]

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Abstract

Of 15 chemicals applied to perennial weeds over the same range of dosages, only five appear to possess a useful toxicity as judged by the number of living plants 12 months after treatment. The effective chemicals can be classified into three groups according to their toxicity, (i) sodium chlorate; (ii) barium chlorate and arsenic pentoxide; and (iii) ammonium thiocyanate and sodium arsenite. The relative toxicity of these three groups of chemicals, judged from the certainly lethal dosage, appears to fall in the proportions of 1 : 1.5 : >2.

Introduction

The relative toxicity of several chemicals to perennial weeds under field conditions was determined in this study. Of the chemicals used, 13 were selected from the 19 substances found to be most toxic to annual weeds in an earlier investigation (2). Two substances less toxic to annual weeds, barium chlorate and α-naphthylamine, were also included, the first because chlorates are generally effective, and the barium salt is less of a fire hazard than the sodium salt (1); and the second because it appears to have a residual toxic effect in the soil (2), a property which may be necessary for the eradication of perennial weeds.

Two series of tests were made: one near Edmonton in 1932 included tests of 11 chemicals on two species, and the other at Saskatoon in 1933 included tests of seven substances on one perennial weed. In the last series several of the earlier tests were repeated so that only 15 different substances were used. These two sets of experiments are subsequently designated the "Edmonton" and "Saskatoon" series respectively.

Materials and Methods

Edmonton Series

The following chemicals were used: sodium hydroxide, sodium arsenite, sodium chlorate, barium chlorate, ammonium thiocyanate, sodium cyanide, zinc chloride, sodium dichromate, phenol, creosote and tar acids, *i.e.*, the acid sludge from oil refineries. Soluble substances were applied as a 10% solution with a hand sprayer, and the creosote and tar acids were used as a 10% suspension and sprinkled on the plants with a watering can. Three dosages of each substance were applied, namely, 700, 750 and 800 lb. of active constituent per acre.

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One set of plots was laid out in a strong stand of couch grass *Agropyron repens* (L.) Beauv. with a few Canada thistles *Cirsium arvense* (L.) Scop., and annual weeds. Adjacent areas 12 ft. square were staked out before treatment. Later, pathways 4 ft. wide were cut out and subsequent observations made on the remaining 100 sq. ft. This procedure excluded the interfering marginal effects resulting from overlapping treatments, or accidentally untreated areas. Each dosage was applied to three plots, and a single plot was treated with each chemical at a rate of 750 lb. per acre, after mowing the weeds.

A less extensive series of treatments was made in the same manner in an area supporting a moderately strong stand of Canada thistle. This series included all the chemicals and dosages listed above, but the greatest and smallest doses were applied to single plots, while the 750 lb. per acre treatment was made in duplicate. Treatments were applied during bright, warm weather in early July. A few evening showers occurred during the period but chemicals were invariably applied to dry vegetation.

Saskatoon Series

These tests included the following chemicals: arsenic pentoxide, sodium chlorate, creosote, tetralin, aniline, α-naphthylamine and tar acids. The first two chemicals are soluble in water and were applied as a 10% solution. As the others are either insoluble, or not sufficiently soluble in water to make a 10% solution, some laboratory studies were made on the emulsification of these materials prior to making the tests. This was felt to be necessary since the results of the Edmonton tests indicated that creosote and tar acids were relatively ineffective, probably because they were applied in rather coarse suspension. These laboratory experiments indicated that a suitable emulsion of creosote could be prepared in 0.2% sodium hydroxide; tetralin and aniline could be emulsified, soap or glue being used as the emulsifying agent, while α-naphthylamine could be dissolved in a small quantity of a solvent (tetralin was used) and emulsified with glue as a stabilizer. The small quantities of these emulsions used in the laboratory tests could be prepared without extremely vigorous agitation, and applied through a spray nozzle. In large containers, however, the emulsions separated into layers of different concentration, and vigorous agitation was necessary before transferring to a sprayer. Most of the emulsions also plugged the spray nozzles rather easily, and to avoid this difficulty a sprinkling can was finally used to make all applications. All chemicals were again applied at three dosages, but as the three rates of application used in the Edmonton series gave essentially similar results, the dosage intervals in these tests were increased, the applications being 600, 750 and 900 lb. per acre of the active constituent.

Plots of the same size as those in the Edmonton series were laid out in a field infested with a vigorous growth of Canada thistle. All dosages were applied to triplicate plots. The treatments were made in the latter part of July, during warm, bright weather, with winds of moderate velocity.

Results

Edmonton Series

The results obtained from the couch grass plots appear in Table I. Three distinct kinds of observations and data were obtained: first, the effect of the chemical on the original herbage; second, the rate of development and character of new plants, and finally, the number of plants per unit area the year following the treatment. The last measurement gives the best estimate of the efficacy of the treatment, because a significant reduction in the number of plants in a treated area, as compared with an untreated one, gives some idea of the true mortality. On the other hand, records taken during the year of treatment give some idea of the rate of killing and the extent of re-growth; but since certain substances can kill the aerial parts

TABLE I

Toxicity of chemicals to couch grass
(Edmonton series)

Chemical	1932 records		1933 record
	Effect on original plants	Effect on development of new plants	No. plants per sq. yd. as % of untreated plots
Sodium chlorate	Slow killing, all dead at freeze-up	Few weak discolored plants	3
Barium chlorate	Slow killing, few stems alive at freeze-up	More new growth than in NH_4CNS or $NaClO_3$ plots	12
Ammonium thiocyanate	Toxic action evident immediately, followed by slow bleaching	Practically no new growth	30
Sodium arsenite	All original herbage killed	Vigorous new growth developed immediately	48
Sodium cyanide	Bleached within two days, apparently completely killed	Considerable new growth	125
Tar acids* (acid sludge)	Almost complete kill	Tall growth of new plants	150
Sodium dichromate	Immediate discoloration and rapid killing	Thick, vigorous new growth three weeks after treatment	155
Phenol	Slow killing, many original stems alive at freeze-up	New growth, rather sickly and thin	170
Sodium hydroxide	Affected plants immediately, most of original growth severely damaged	New growth appeared early and developed luxuriantly	175
Creosote*	Immediate wilting but plants made good recovery, only partial kill resulted	—	187
Zinc chloride	Slow killing of most of original herbage	New growth of rather tall weak plants	246

Difficult to apply satisfactorily.

of the plant without seriously affecting the roots, it is impossible to estimate the permanent effect from such observations. It was found that whenever a chemical failed to kill the original herbage completely there appeared to be less tendency for new growth to appear, but owing to the large number of living plants in evidence under these conditions it is difficult to establish this point.

In no case was there any detectable difference between the different rates of application or between the block of mowed and unmowed plots. Observations and counts were made in all plots but these are grouped or averaged for all doses of the same material in Table I. The weed counts made in 1933 were taken at approximately three-week intervals in the months of May and

TABLE II

TOXICITY OF CHEMICALS TO CANADA THISTLE
(Edmonton series)

Chemical	1932 records		1933 record
	Effect on original plants	Effect on development of new plants	No. plants per sq. yd. as % of untreated plots
Sodium chlorate	Slow killing, all dead by freeze-up	Few weak, sickly plants	0
Barium chlorate	Slow killing, all dead by freeze-up	Few weak, sickly plants	0
Ammonium thiocyanate	Slow killing, all dead by freeze-up	Few weak, sickly plants	50
Sodium arsenite	Original herbage apparently partly killed, some recovery	Considerable new growth	120
Sodium cyanide	Original herbage killed	Profuse new growth	170
Tar acids* (acid sludge)	Only partial destruction and good recovery	New growth very uneven, apparently normal plants interspersed with few small sickly ones	110
Sodium dichromate	Complete destruction	Plentiful new growth appeared shortly after treatment, some of these plants died subsequently	110
Phenol	Complete destruction	Plentiful growth appeared late in season	120
Sodium hydroxide	Rapid and complete destruction	Plentiful growth, some showing signs of weakness	82
Creosote*	Injured upper portion of plant only, recovery from unaffected growth below	Plentiful new growth appeared normal	120
Zinc chloride	Rapid killing but probably incomplete destruction	Luxuriant growth of new plants	110

**Difficult to apply satisfactorily.*

June. These usually increased, in the plots unaffected by treatment, as the season advanced, but they were again averaged in reducing the data. Since the plants were counted three times on four square-yard areas, chosen at random in each plot, and as ten plots were treated with each chemical, the average figure presented in the last column of Table I is the result of 120 counts. Statistical analysis showed that the first three chemicals reduced the number of weeds significantly as compared with the untreated check, while the fourth, sodium arsenite, was just on the border line of significance. All the other treatments increased the number of plants as compared with the untreated plots, but most of these differences are not statistically significant.

The results of similar treatments on Canada thistle appear in Table II, the chemicals being arranged in the same order as in Table I. Here also there was no detectable difference between the three rates of application, and the results on all plots were again grouped and averaged. In 1933 the number of plants per square yard was counted as before, three times at three-week intervals, but as there were only four plots of this weed treated with each chemical, the numbers in the last column are the average of only 48 counts. The two chlorate salts killed all the plants, and statistical analysis showed that ammonium thiocyanate reduced the number of weeds significantly. With the exception of sodium cyanide, where stimulation was evident, none of the other treatments differed significantly from the controls.

Saskatoon Series

The results obtained in the Saskatoon experiments appear in Table III, the figures representing the average results from triplicate plots. More detailed records were taken during the year of treatment, the number of dead leaves, stems, and new plants being counted one day, one week and one month after treatment, followed by a final observation made just before freeze-up. These results obtained at the different rates of application appear separately, as the larger intervals between doses gave significantly different results in some instances.

It can be seen from Table III that the majority of the chemicals killed most of the leaves and stems on the original plants. No new plants were evident in any of the plots one week after treatment, but subsequent observations showed considerable growth, except in the plots treated with sodium chlorate and arsenic pentoxide, where only a few plants developed.

In 1934 detailed records were again taken on all plots, but as sodium chlorate and arsenic pentoxide were the only effective chemicals, the results are merely reported in a descriptive form in Table III. A dosage of 600 lb. per acre appears to be about the certainly lethal dose of sodium chlorate to Canada thistle under the conditions of this experiment, while about 900 lb. of arsenic pentoxide is required to produce complete mortality. It is likely that smaller dosages of these chemicals would effect a useful, though incomplete, mortality.

TABLE III

TOXICITY OF CHEMICALS TO CANADA THISTLE
(Saskatoon series)

Chemical	Dosage, lb. per acre	Average number plants per sq. yd.	1933 records—Dead leaves and stems and number of new shoots per sq. yd. at intervals after treatment										1934 records
			One day		One week		One month			Before freeze-up			
			Dead leaves, %	Dead stems, %	Dead leaves, %	Dead stems, %	Dead leaves, %	Dead stems, %	New shoots, No. per sq. yd.	Dead leaves, %	Dead stems, %	New shoots, No. per sq. yd.	
Sodium chlorate	600	30.6	80	16.6	100	55	100	96.6	2.6	99.6	98.3	1	No growth
	750	31	95	25	100	80	100	98.3	1	100	100	0	No growth
	900	32.6	100	28.3	100	91.6	100	100	0	100	100	0	No growth
Arsenic pentoxide	600	30.3	100	28.3	100	96.6	100	100	0	100	100	0	A few sickly looking thistles. Many wild barley seedlings
	750	30.3	100	40	100	98.3	100	100	0	100	100	0	No thistle but some wild barley
	900	30.3	100	38.3	100	100	100	100	0	100	100	0	No growth
Acid sludge	600	26.6	100	85.5	100	100	100	100	38	100	100	44.3	Thick and luxuriant growth
	750	31.3	100	88.3	100	100	100	100	47	100	100	59.3	
	900	32	100	96.3	100	100	100	100	46	100	100	57.3	
Alpha-naphthylamine	600	26	73	0	100	31.6	100	83	15.6	100	96.6	31	Thick healthy growth of the weed and grasses
	750	26	83.6	0	100	43.3	100	96.6	30 3	100	100	43.6	
	900	29.6	83	0	100	55	100	100	24	100	100	47.6	
Aniline	600	20	61.6	10	90	63.3	90	83.3	1.6	90	83.3	9.6	Normal stand of the weed and grasses
	750	20	70	10	96.6	81.6	96.6	96.6	3.3	96.6	96.6	16.6	
	900	21.6	86.6	13.3	100	96.6	100	100	7	100	100	26.6	
Tetralin	600	16.3	11.6	0	33.3	0	33.3	0	3.6	33.3	0	4.6	Norma stand of the weed and grasses
	750	20	16.6	0	51.6	0	51.6	0	5.6	51.6	0	7.6	
	900	22.3	21.6	0	56.6	0	56.6	4.6	4.3	56.6	4.6	8	
Creosote	600	28.6	6.6	0	23.3	0	35	0	10	36.6	0	13.3	Normal stand of the weed and grasses
	750	29	11.6	0	41.6	0	55	0	15.6	55	0	19.6	
	900	30.6	18.3	0	45	0	65	8.3	20.6	68.3	8.3	20.3	

A supplementary experiment was carried out to determine the residual toxicity of the two effective chemicals in the soil 12 months after treatment. This was done by determining the viability of the Canada thistle roots extending into the treated plots from the adjacent untreated areas. The condition of the roots in successive two-inch layers is given in Table IV. It is evident

TABLE IV

RESIDUAL EFFECT OF CHEMICALS IN SOIL TWELVE MONTHS AFTER TREATMENT, JUDGED FROM VIABILITY OF CANADA THISTLE ROOTS EXTENDING INTO TREATED PLOTS FROM UNTREATED AREAS

(Saskatoon series)

Depth examined	Condition of roots	
	Sodium chlorate plots	Arsenic pentoxide plots
In.		
0 – 2	Dead	Dead
2 – 4	Dead	Dead
4 – 6	Dead	Mostly fresh
6 – 8	Dead	
8 – 10	Dead	Fresh
10 – 12	Alive, but injured	
12 – 14	Fresh	

that sodium chlorate was still present in sufficient quantity in the first 10-in. layer of soil to kill roots penetrating that layer. In the plots treated with arsenic pentoxide this toxic condition of the soil was observed only in the first four inches. The most significant point is that both the effective chemicals had rendered the soil sufficiently toxic to prevent growth in the surface layer 12 months after treatment. Effects of this sort would of course be expected to vary with the character of the soil and rainfall.

Discussion and Conclusions

These results are of interest in connection with the methods employed for estimating the efficacy of perennial herbicides under field conditions. It has already been pointed out (3) that different investigators have made their final observations on the treated areas at various times after treatment. Some judge the efficacy shortly afterwards, others after longer periods, but during the same growth season, and still others have based their conclusions on the condition of the treated area a year or so later. It would appear that, until more information is available, the last is the only reliable criterion. The results presented in the foregoing tables show that some chemicals kill the herbage slowly, while others cause immediate death. The apparent mortality in such cases is therefore a function of time, and observations made within a month or two of treatment may lead to erroneous conclusions, since some of the slow-killing chemicals have the greatest permanent effect. Again two substances may kill the original herbage, but one may allow new growth to develop while the other may not. Judging from the results obtained in these experiments a chemical that kills the original herbage and does not allow

new growth to develop or survive during the season of treatment, will generally be found reasonably effective, as judged by the number of plants present the next season. However, a period of at least two months must elapse in order to permit new growth to develop.

The results show that a substance of high inherent toxicity may kill the aerial parts of the plant without having any significant effect on the roots. The experiments at Saskatoon indicate that the death of the roots depends on the presence of the chemical in the soil, and if the chemical is detoxicated by the soil before the roots are dead, the treatment is ineffective.

Of the 15 substances tested, only five appear to have a useful toxicity, namely, sodium chlorate, barium chlorate, arsenic pentoxide, ammonium thiocyanate and sodium arsenite. Sodium chlorate was most toxic, the certainly lethal dose (C.L.D.) under the conditions of these tests being about 600 lb. per acre. Barium chlorate and arsenic pentoxide are probably equally toxic, the C.L.D. being about 900 lb. per acre. Ammonium thiocyanate and sodium arsenite are less toxic than the others, but the C.L.D. cannot be given from the results obtained although it is probably in excess of 1200 lb. per acre. The relative toxicity of the effective chemicals, as judged from the C.L.D. is, therefore: sodium chlorate : barium chlorate and arsenic pentoxide : ammonium thiocyanate and sodium arsenite : as $1 : 1.5 : >2$. It is possible that some of the other substances would effect mortality at much higher dosages, but unless they can be obtained at very low cost, they would not be practical herbicides. The results do not permit any statement to be made with respect to the relative susceptibility of the two species to applied chemicals.

References

1. Cook, W. H. Can. J. Research, 8 : 509-544. 1933.
2. Cook, W. H. Can. J. Research, C, 15 : 299-323. 1937.
3. Cook, W. H. and Halferdahl, A. C. National Research Council, Ottawa. Bulletin 18. 1937.

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THE TAXONOMY OF *DIPHYLLOBOTHRIUM LATUM* (LINNÉ, 1758) IN WESTERN CANADA[1]

BY ROBERT ARNOLD WARDLE[2] AND ELLEN LOUISE MCCOLL[3]

Abstract

On the basis of accepted criteria for the differentiation of species of *Diphyllobothrium* Cobbold, diphyllobothriid material from humans and dogs in Manitoba, one of the three endemic areas of diphyllobothriosis in North America, is adjudged to be homogeneous and to be specifically identical with authenticated *D. latum* material from Russia, Finland and Japan. The classificatory value of various characteristics of *D. latum* and the extent of variability of these characteristics in Canadian material are discussed.

Introduction

In spite of a long and intimate sociological association, the physiological differences between man and dog are sufficiently profound to discourage the common possession of identical species of parasites. *Diphyllobothrium latum* (Linné, 1758) is, with the possible and doubtful exception of *Dipylidium caninum*, the only tapeworm found commonly and habitually in both dog and man, and if we except a few forms of restricted distribution that stray to man occasionally from the dog, it is the only parasite, external or internal, common to both dog and man and apparently equally at home in either host.

It is exceptional also among tapeworms in its wide range of definitive hosts, a range that includes at least seven species of *Felis*, four species of *Canis*, four species of seal, three of bear, one of whale, mongoose, fox, mink, domestic pig and man.

Such catholicity of host adoption, when shown by a member of a group of parasites notoriously restricted in their choice of definitive hosts, should raise the suspicion that the characters chosen arbitrarily for the delineation of this species are inadequate, and that *latum* is in fact not a species at all but a group of closely related physiological species in course of evolutionary divergence. The point has not been raised previously, to our knowledge, and no close comparison appears to have been made between alleged *latum* specimens from different host species within a restricted area.

Relatively common in sled dogs in certain districts of Manitoba is a form of tapeworm sufficiently resembling the accepted descriptions of *latum* to justify identification with it (4, 5, 9, 12). It is not uncommon also among

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ranch-raised foxes and mink. To what extent it occurs among wolves, bears and wild foxes is undetermined; nor is its distribution among the human population accurately known. Fecal examinations of hospital patients, university students, reservation Indians, and so forth, suggest an extremely low incidence of infestation, probably not exceeding 2%. Apart from people of Indian blood, the population is predominantly French, Scottish, Ukrainian, Icelandic and Jewish in ancestral origin, and the consumption of raw fish is not a common custom. A few cases of human diphyllobothriosis are noted each year, however, by medical practitioners, chiefly among Jewish housewives, and there is reason to attribute some of the cases recorded in other cities of North America to initial infection from fishes of Manitoba origin.

Taxonomic Criteria

The validity and limitations of the characters used in distinguishing species of tapeworms have been discussed previously (10). For the present comparison the characters selected as being least likely to be influenced by variations of the host environment were the uterine shape, the position of the common genital pore, and the "height", *i.e.*, the dorso-ventral measurements in a 16μ-thick transverse section of a gravid proglottis, of the cuticle, subcuticle, cortex, vitellaria, muscle zone, medulla, testes and cirrus sac, as compared with the height of the proglottis itself. Observations were also made upon the shape and size of the scolex, strobila and proglottids.

The material, comprising specimens from humans in Manitoba, northern Russia, Finland and Japan, and from dogs in Manitoba and northern Russia, had been fixed in formalin, and for purposes of microscopical examination was stained by Delafield's haematoxylin technique, dehydrated in dioxan, cleared in methyl salicylate and mounted in xylol-balsam.

Scolex and Strobila

The scolex of *Diphyllobothrium latum* is described by European authors as spoon-shaped (spatulate), almond-shaped (amygdalate) or olive-shaped (olivate), and approximately 2 mm. long by 1 mm. in maximum breadth.

The shape and size of the scolex and strobila of a living tapeworm just removed from the host and observed in an artificial medium are continually altering owing to the absence of skeletal tissue. The extent of the change is determined by the chemical nature, hydrogen ion concentration and temperature of the medium, and by the length of time that the tapeworm has been divorced from its host. This question has already been discussed (9, 11), but the point may be stressed that even if the fixative employed were to kill the tapeworm instantaneously, which in actual practice never happens since the action of the fixative is impeded by the cuticle, no two specimens will be identical in shape and size after being killed, even if approximately so when alive. Precise descriptions of the shape and size of the scolex and strobila are therefore unreliable. On the other hand, the post-mortem shape and dimensions are determined by the extent of muscular relaxation at death,

so that, among a range of specimens killed in the same way by exposure to a weak fixative such as 5–10% formalin, it should be possible to determine the limiting boundaries of shape and size, since the extent of muscular relaxation will vary only within certain limits.

Among the specimens obtained from dogs and foxes in Manitoba,—approximately 100 specimens—the shape of the scolex varied from narrowly lanceolate to broadly spatulate (Fig. 1). A similar range of shape was seen

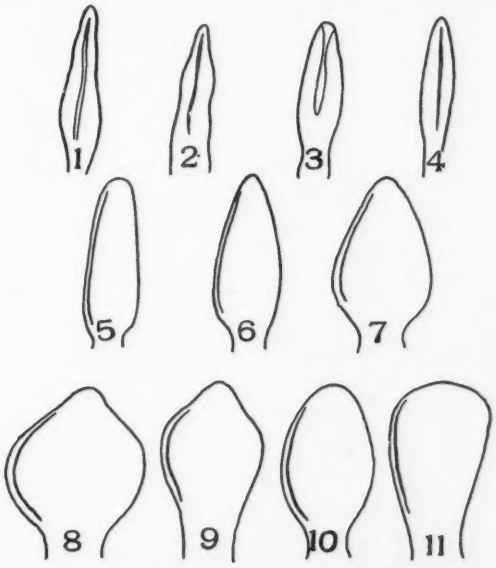

FIG. 1. *Range of scolex shape of Diphyllobothrium latum in Manitoba material. 1, 2, 3, 4 scolices less than 25 hours old; 5, digitate; 6, lanceolate; 7, ovate; 8, cordate; 9, clavate; 10, olivate; 11, spatulate.*

among collections of 20–27 scolices from individual dogs. The commonest type among local diphyllobothriid material is the clavate (Fig. 2), with its maximum width at one-third of the length from the bluntly rounded apex of the scolex. There is a tendency, probably correlated with a progressive deterioration of the scolex musculature, for the shape of the scolex to change, as the worm ages, from an active lanceolate or digitate type to a relatively inactive clavate or spatulate type.

Among 29 scolices taken from a dog infested for 75 days, the lengths varied, after formalin fixation, from 1.326–2.400 mm. with a mean value of 1.700 mm.; the breadths varied from 0.625–1.000 mm. (maximum breadth accompanying minimum length) with a mean value of 0.747 mm. Among 24 scolices from a dog infested for 56 days the mean length by breadth values were 1.523×0.926 mm. In one scolex from a Russian dog, infested 36 days, the shape was clavate, the dimensions 1.25×0.75 mm. Unfortunately only

four scolices from man were available. Two Russian scolices were spatulate, with mean dimensions of 1.5 × 0.75 mm. Two scolices from man in Manitoba were clavate with mean dimensions 1.25 × 0.9 mm. Magath (3) describes one scolex from man in Minnesota as 1.4 × 0.5 mm. in dimensions, and figures it as midway between digitate and olivate.

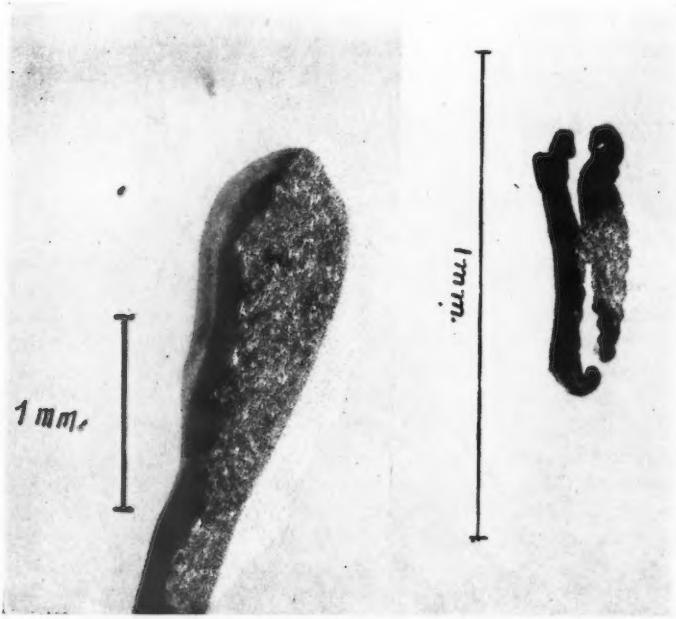

FIG. 2. *Scolex of Diphyllobothrium latum from cat, Winnipeg, seen in surficial view and in transverse section.*

The shape of 27 scolices removed from the host (kitten) within 25 hr. after initial infection was invariably acutely lanceolate (Fig. 1) and the mean dimensions 1.66 × 0.5 mm. Presumably, therefore, no growth of the scolex takes place after the plerocercoid larva reaches the definitive host. This appears to be the case in tapeworms generally. The scolex is necessary to anchor the small and muscularly weak early strobila, but in the older strobila it serves little purpose, the tapeworm maintaining its station by muscular tonus.

The neck region extends from the posterior margins of the bothria to the first interproglottid boundary. Although the most important physiological region of the tapeworm, it is histologically the most obscure, since it does not react with the usual microtechnical reagents. Its posterior margin—the first interproglottid groove—is difficult to fix with precision, so that the extent to which the neck region corresponds with the original plerocercoid body is

uncertain. Among the 27 individuals up to 25 hr. old, mentioned above, the lengths—including the scolex—varied from 6–24 mm., with a mean of 10.0 mm. Segmentation begins usually at about 25 hours after initial infection. Among five 30-hr. strobilae, whose mean length was 45 mm., the neck region varied from 3.5–4.0 mm. in length and can represent therefore only the anterior third of the plerocercoid. Among 23 specimens from a dog infested 56 days, the neck lengths varied from 1.4–2.6 mm. with a mean value of 2.2 mm. Magath (3), however, estimated the neck length of a single specimen from man as 8 mm.

Segmentation of the early strobila between 25 and 72 hr. old appears almost simultaneously along the whole strobilar length, the primary strobila being thus formed as in other families of pseudophyllidean tapeworms—Ptychobothriidae, Amphicotylidae—where no neck is present. The absence of a neck from certain diphyllobothriid species parasitic in pinnipede Carnivora and ascribed to the genus *Diphyllobothrium* Cobbold would suggest that this method of primary strobila formation was the original method in *Diphyllobothrium* also and that the neck is, from an evolutionary point of view, a new structure. It would be a matter of great interest to know whether, in such apparently primitive diphyllobothriids, the secondary strobila is formed as in Pseudophyllidea generally, by division of pre-existing proglottids or, as in *Diphyllobothrium latum*, by origin of new proglottids from the neck region.

The early strobila is completely segmented by the 72nd hour after initial infection. It consists of "long" proglottids, *i.e.*, proglottids with a length of 1.5–2.0 times the breadth. The proglottids are markedly craspedote and the terminal proglottis has a characteristic bluntly rounded posterior margin. In superinfested dogs such primary strobilae may be found several months after initial infection. The alleged species *parvum* of Stephens (7) appears almost certainly to have been a primary strobila of *latum* about nine metres in length, occurring in man, but under the conditions of light infestation such as occur usually in human hosts it is probable that the primary strobila passes fairly rapidly into the secondary phase.

The new proglottids of the secondary strobila are at first linear, much wider than long; as they mature they become quadrate; under conditions of ample nutrition they remain linear, the breadth being three to four times the length. Under conditions of superinfestation in dogs the characteristic shape of the secondary proglottids is quadrate, or nearly so, but strips of long and craspedote proglottids may interrupt the sequence of quadrate proglottids. Such recurrence of primary strobilar characters may arise from a slowing down of genital development and a release of material for tissue growth. Under conditions of light infestation and ample nutrition the proglottids are linear, because the nutritional demands of the reproductive system limit the surplus of material available for tissue growth. It is probable that a survey of tapeworms in general would show that when the number of individuals per host is habitually low, in consequence of premunition, and the sexual development rapid, the proglottid shape is usually linear.

The most extensive data concerning the size of the strobila in *D. latum* in Europe are those of Tarassow (8) who found that, among 405 specimens from humans in the Karelia-Murmansk-Leningrad districts of U.S.S.R., the strobilar length varied from one to seventeen metres, the majority of the specimens being 8-10 metres in length. Tapeworm material obtained from human hosts by vermifugation is usually incomplete, and accurate measurements of strobilae from North American human hosts are not available. Scattered observations suggest that they rarely exceed two metres in length. In dogs they are smaller still. In superinfested dogs in our experiments the mean strobilar length varied from 25.6 cm. (32 days after initial infection) to 169 cm. (98 days after initial infection).

On the whole, therefore, variations in shape and size of the scolex and strobila of *Diphyllobothrium latum*, although of considerable physiological interest, have little taxonomic value.

Internal Dimensions

There has been a tendency in recent years for students of tapeworm taxonomy to provide meticulous micrometric measurements of such internal structures as the cirrus sac, testes, germarium, vitellaria and genital ducts, and exhaustively detailed descriptions of the cuticle, subcuticle, musculature, parenchyma and so forth. Valuable as such ponderous and scholarly descriptions must be to the student of comparative histology, it may be doubted whether they are of like value to the taxonomist, although certainly preferable to the inadequate descriptions that have accompanied the announcement of many alleged new species. A tapeworm has no system of skeletal tissues such as serve in other animals to keep the dimensions of anatomical structures between definable limits, and such structures as the cirrus sac, uterus, and genital ducts have markedly contractile muscular walls that make them extremely susceptible to the distorting effects of technical reagents. A tapeworm killed quickly by suspension in a hot aqueous medium such as 10% formalin or corrosive sublimate-acetic acid, or even hot water alone, will increase in length by 50–60% owing to the relaxation of the longitudinal musculature. Absolute dimensional measurements of tapeworm structures are therefore of doubtful value unless the material under comparison has received identical treatment. On the other hand, relative measurements where the length or breadth or height of the proglottis is used as a standard of comparison have considerable value when applied to a range of material killed and preserved in like fashion.

The data in Table I are mean values of the height, *i.e.*, the vertical diametrical measurements, of the cuticle, subcuticle, cortex, vitellaria, muscle zone, medulla, testes and cirrus sac, expressed as percentages of the height of the proglottis measured at a point midway between the middle line and the lateral margin, except in the case of the cirrus sac whose height is compared with the height of the proglottis in the middle line.

TABLE I

DORSO-VENTRAL DIMENSIONS OF PROGLOTTIS STRUCTURES EXPRESSED AS PERCENTAGES OF THE PROGLOTTIS THICKNESS. LENGTH, BREADTH AND THICKNESS OF THE PROGLOTTIS ARE GIVEN IN MILLIMETRES

Origin	Lgth.	Bdth.	Th.	Cut.	Sc.	Cort.	Vit.	Musc.	Med.	Cs.	Test.
Man, Russia	4.18	10.0	0.700	0.7	4.0	24.0	14.0	7.0	30.0	0.66	21.0
Man, Finland	2.90	8.0	.760	0.4	8.0	14.0	11.0	16.0	21.0	.47	15.0
Man, Japan	3.22	10.0	.661	1.06	6.8	15.0	10.6	16.0	22.7	.43	16.4
Mean values				0.72	6.2	18.0	12.0	13.0	24.6	.52	17.5
Man, Winnipeg,	2.47	7.5	.856	0.94	6.0	23.5	9.4	11.7	17.6	.50	14.0
Man, Winnipeg	3.54	10.0	.590	0.34	8.5	11.0	13.6	22.0	33.2	.51	24.6
Man, Winnipeg	1.97	7.0	.174	0.28	7.0	16.7	9.8	15.4	21.0	.57	18.1
Mean values				0.52	7.2	17.1	11.0	16.3	24.0	.53	19.0
Dog, Russia	5.15	5.0	.850	1.17	4.7	27.5	11.7	11.7	17.6	.75	17.6
Dog, Churchill	1.05	3.0	.978	1.40	12.0	14.3	12.2	14.3	21.1	.31	13.8
Dog, Winnipeg	7.87	6.5	.540	2.80	11.1	14.8	11.1	3.7	22.2	.60	14.0
Mean values				1.79	9.3	19.5	11.0	9.9	20.3	.55	15.1

Cut. cuticle, Sc. subcuticle, Cort. cortex, Vit. vitellaria, Musc. muscle zone, Med. medulla, Cs. cirrus sac, Test. testes.

The material compared comprises three specimens from man in Manitoba, three from man in Leningrad, Finland and Japan, and three specimens from dogs in Manitoba (Winnipeg, Churchill) and Leningrad. The material in each case was fixed in formalin, embedded in paraffin, cut to a thickness of 16μ and stained by the iron-haematoxylin technique.

Comparison of data from human material suggests that, despite considerable individual variation in the percentage proportions of the internal structures measured, there is a fundamental similarity, brought out particularly by comparing the mean values of the three foreign specimens with those of the Manitoba specimens. The chief differences are in thickness of cuticle, which might be expected to vary with host gut conditions, and thickness of the muscle zone, which might be expected to vary with the degree of relaxation induced by fixation. Among the specimens from dogs there was greater variation, especially as regards the cuticle, subcuticle and muscle

zones, but again the mean values of the three do not depart so widely from those of human material as to suggest any specific differentiation. The material from Churchill, the most northerly location for *D. latum* yet recorded in Canada, consisted of twelve strobilae, believed to be primary, with a mean length of 30 cm. and width of 3 mm. This material showed certain peculiarities, notably the markedly digitate scolices, 1.7 × 0.7 mm. in dimensions, the great proglottid thickness (978μ), extremely thick subcuticle (90μ), thick muscle zone (140μ), small cirrus sac, small testes—scarcely larger than the vitellaria—, with long axes vertical instead of horizontal, and the absence of circum-cloacal papillae which are usually very prominent in material from dogs. In the absence of human material from this area, however, it would be premature to draw any conclusions as to specific or sub-specific distinction concerning this material.

Genital Pore

The descriptions of the genitalia of *D. latum* provided by early authors have been incorporated and added to in the accounts given by Sommer and Landois (6) and by Magath (3) and need not be repeated here.

The term "porus genitalis" is not clearly defined by earlier authors and actually in *latum* there are three such pores, the vagina and cirrus sac opening separately into a genital cloaca which itself has an exit to the exterior. The

TABLE II

LOOP COUNTS, EACH SIDE OF THE MIDDLE LINE, FOR THE ANTERIOR UTERUS (Ant. ut.) AND POSTERIOR UTERUS (Post. ut.) AND RATIOS OF DISTANCE (Gp.) OF COMMON GENITAL PORE FROM ANTERIOR PROGLOTTID MARGIN TO PROGLOTTID LENGTH (Lgth.). THE VALUES ARE MEAN FOR *n* CONSECUTIVE PROGLOTTIDS

Origin	*n.*	Ant. ut.	Post ut.	Lgth., mm.	Gp., mm.	Ratio
Man,						
Russia	20	5.2 : 4.7	2.05 : 2.55	5.67	1.35	0.234
Russia	20	5.9 : 5.5	2.80 : 3.20	4.18	0.857	.205
Finland	20	6.3 : 6.0	1.80 : 2.30	2.90	0.417	.144
Japan	20	4.7 : 4.3	1.40 : 2.00	3.22	0.825	.256
Manitoba	20	4.2 : 4.5	1.30 : 1.50	2.47	0.399	.162
Manitoba	10	4.1 : 4.4	2.40 : 3.40	3.54	0.810	.229
Manitoba	20	4.2 : 4.5	2.70 : 2.90	1.97	0.386	.196
Manitoba	20	3.8 : 3.4	1.30 : 2.00	1.70	0.308	.181
Manitoba	20	3.9 : 3.4	1.10 : 1.70	1.38	0.252	.182
Manitoba	20	4.9 : 5.1	1.90 : 2.20	2.07	0.330	.159
Manitoba	20	4.9 : 5.2	2.00 : 2.50	3.03	0.635	.209
Manitoba	20	5.0 : 4.7	— : —	1.70	0.312	.184
Dog,						
Russia	20	7.2 : 7.3	2.9 : 2.7	5.15	1.210	.235
Gimli	12	5.6 : 6.3	1.5 : 1.3	2.30	0.308	.134
Winnipeg	7	8.0 : 7.9	3.1 : 3.9	7.87	2.012	.255
Winnipeg	12	6.2 : 6.0	2.8 : 2.4	1.60	0.324	.202
Churchill	9	4.0 : 4.0	— : —	1.05	0.184	.175
Fox						
Gimli	20	5.3 : 5.6	2.7 : 2.6	3.06	0.572	.187

term will be used here to imply the central point of the common genital pore or cloacal aperture. This is frequently placed vertically ventral to the male pore but occasionally, due to distortion of the cuticle, it is shifted to the female pore region. Its position is usually described in terms of its distance from the anterior margin of the proglottis and is a favorite taxonomic character with students of Diphyllobothriidae. In European specimens it is usually said to be situated at about the anterior quarter of the proglottid length. In the case of a Minnesota specimen Magath (3) gives a distance of 0.44 mm. from the anterior margin of a segment 2.5 mm. in length, that is to say a ratio to proglottid length of 0.18.

Table II gives this ratio of genital pore position for a series of consecutive proglottids, usually 20, in material from man in Russia, Finland, Japan, Manitoba (eight specimens) and from dogs in Russia and Manitoba (Winnipeg, Gimli, Churchill). In the material as a whole the ratio varied from 0.134 (Gimli dog) to 0.255 (Winnipeg dog) with a general mean of 0.196. In Manitoba human material the ratio varied from 0.162–0.229 with a mean of 0.188; in Manitoba dog material it varied from 0.134–0.255 with a mean of 0.19. The material from a fox had a ratio of 0.187; from a dog at Churchill, a mean ratio of 0.175. On the whole therefore the common genital pore in Manitoba material is nearer the anterior margin of the proglottis than it is in Russian or Japanese material, but the difference of position is too slight to constitute a valid specific differentiation.

Uterine Shape

The uterus of *Diphyllobothrium latum* is a tube fixed between its entrance from the oötype and its exit to the exterior, and thrown into a series of coils or "loops". The number of these loops on each side of the middle line has long been a taxonomic character for species of *Diphyllobothrium*. The steps in the development of this looped condition have not to our knowledge been described and may be briefly discussed here.

In a specimen from man in Leningrad, the anlage of the uterus first appears at 50 mm. from the scolex tip, that is to say between the 250th and 300th proglottids. In the next 156 proglottids, a distance of 165 mm., the uterus appears as a straight, faintly staining structure, presumably tubular, with the anterior end swollen and bent ventrad upon itself (Fig. 3, A) although the apparent swollen extremity may be the anlage of the cirrus sac. By elongation through growth, the straight uterus becomes a loose corkscrew which persists through the next 84 proglottids (Fig. 3, B). Continued growth brings about a lengthening of the corkscrew coils, producing a zigzag pattern that persists through the next 80 proglottids (Fig. 3, C). As eggs begin to pass from the oötype into the uterine tube the somewhat angular bends become rounded, closer together, loop-like and approximately parallel, but directed slightly dorsad and ventrad from the horizontal plane. A distinction now appears between an anterior set of loops—large, approximately parallel, non-stainable, containing eggs, each with a single, longitudinal

wrinkle—and a posterior set of loops—small, irregularly arranged, stainable with haematoxylin, containing eggs whose shell, in mounted specimens, is usually intact or occasionally in a condition of general collapse. The wrinkling of the eggs is due to dehydration by the reagents used in microtechnical preparation. In weak formalin or in water no such wrinkling is shown by extruded eggs; in alcohol of 70% or greater strength, a single longitudinal wrinkle appears immediately. Eggs in the posterior uterus appear to have no operculum, whereas those in the anterior uterus are operculated; the distortion of the egg shell observed among the latter eggs seems in some way to be correlated with the presence of the operculum.

The loops of the posterior uterus are difficult to count, often distinguishable only with difficulty, and although used by Faust *et al.* (1), are commonly ignored by systematists.

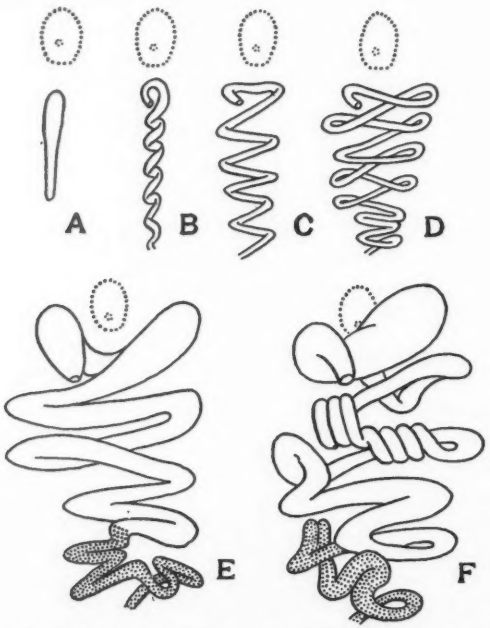

FIG. 3. *Stages in the development of the uterus of Diphyllobothrium latum. The shaded area indicates the posterior uterine region.*

The primary uterine pattern is thus a series of parallel and lateral loops, whose anterior members have thin, chitinoid and non-stainable walls enclosing eggs wrinkled longitudinally (in mounted specimens), and whose posterior members have thicker, epithelioid, stainable walls enclosing eggs that are either not distorted or else occasionally in a condition of general collapse. If this primary pattern persisted through the whole strobila the uterine loop

count would be a relatively stable and easily estimated taxonomic character. Unfortunately, the difficulty of loop counting is greatly increased by egg elimination—exhausted proglottids are almost impossible to use for loop counts—,and by distortion induced by egg pressure within the uterus.

In the primitive, long type of proglottis, the anterior loop count is approximately eight on either side of the middle line, the posterior count approximately three or four on either side. However, the later quadrate and linear proglottids show a reduction from these numbers, and their uterine loops, owing to the intrinsic pressure of the increasing number of eggs, aided by the extrinsic pressure of the muscular medullary margin, lose their parallel alignment and lie at an angle to the central area so that a rosette-like arrangement of the loops is produced. This is especially marked in the posterior uterine region. The bends of the loops become bloated with eggs, and in mounted preparations may give the appearance of a linear series of egg sacs on either side of the middle line. The two most anterior bends are particularly enlarged. Continued pressure brings about a corkscrew-like torsion of some of the anterior loops, or a sub-looping, so that in flattened preparations they may appear forked. Russian material shows this pseudo-forking to a marked degree; Manitoba material shows more of the torsion effect. If a fork be counted as two loops, then the loop counts of European material will appear greater than those of Manitoba material. The use of uterine loop counts as a taxonomic character is therefore not altogether satisfactory. The count is difficult to estimate with accuracy and the values found vary within relatively wide limits.

Anterior and posterior loop counts—each the mean of 20 consecutive proglottids—are given in Table II for material from various sources. It will be noted that the Manitoba human material agrees fairly well in having approximately 4.3 anterior loops and approximately two posterior loops on each side. The single Japanese specimen agreed on the whole with the Manitoba material. The Russian and Finnish material, owing to the prevalence of forked loops, showed higher mean counts of 5.6 for anterior and 2.5 for posterior loops. Material from dogs, both Russian and Manitoban, showed higher mean counts, the mean being 6.5 for anterior, 2.6 for posterior. For the material as a whole, excluding the Churchill dog material, the mean anterior count was 5.3, the posterior count 2.3.

The influence of secondary distortion upon the uterine loop counts is shown in Table III which gives the results of loop counts of 150 consecutive proglottids of Russian human material and 70 consecutive proglottids of Manitoba human material. To condense the table, only the means of each set of ten proglottids are given. It will be noted that in Russian material where secondary pseudo-forking is marked and the forks are counted as single loops, the uterine loop counts increase apparently from 3.3 : 3.2 in the anterior uterus, and 1.2 : 1.16 in the posterior uterus, to 5.5 : 5.2 : in the anterior uterus and 2.0 : 2.7 in the posterior uterus respectively, whereas in Manitoba material, where such pseudo-forking is rare and distortion produces chiefly

TABLE III

UTERINE LOOP COUNTS FOR CONSECUTIVE PROGLOTTIDS OF THE ONE STROBILA. THE VALUES REPRESENT MEANS OF SUCCESSIVE SETS OF TEN PROGLOTTIDS

Russian (human)		Manitoba (human)	
Anterior uterus	Posterior uterus	Anterior uterus	Posterior uterus
3.3 : 3.2	1.2 : 1.6	3.4 : 3.1	0.5 : 0.8
3.0 : 3.0	1.4 : 1.3	3.2 : 3.5	0.8 : 0.6
3.3 : 3.5	1.6 : 1.9	3.3 : 3.2	0.7 : 0.9
4.2 : 4.2	1.9 : 1.9	3.0 : 3.3	0.2 : 0.7
3.9 : 3.8	1.6 : 2.2	3.6 : 4.1	0.9 : 1.0
4.2 : 4.6	1.9 : 1.7	3.8 : 3.7	0.7 : 1.0
4.4 : 4.3	1.7 : 2.0	3.7 : 3.5	1.1 : 0.7
4.5 : 4.5	1.5 : 2.2		
4.3 : 4.5	1.9 : 2.4		
5.0 : 4.9	1.8 : 2.4		
5.2 : 4.6	1.5 : 2.5		
5.3 : 5.8	1.9 : 2.2		
5.2 : 5.5	2.3 : 2.0		
5.6 : 5.3	2.0 : 2.1		
5.5 : 5.3	2.0 : 2.7		

spiral twisting of the loops, the anterior uterine counts vary only between 3.4 : 3.1 and 3.7 : 3.5, the posterior uterine counts vary only between 0.5 : 0.8 and 0.7 : 1.0.

Conclusions

If we accept as valid criteria of specific distinction the characteristics of *Diphyllobothrium latum* discussed above, there does not appear to be sufficient difference between Manitoba human and canine material, Manitoba and Russian human material, Manitoba and Russian canine material, to suggest that more than one species is involved. There are however certain slight but constant differences between Manitoba and foreign material, and the Manitoba material may be defined as: *Diphyllobothrium* sp., probably *latum*, with scolex lanceolate to spatulate in shape but commonly clavate; with proglottids linear in human material but quadrate or even longitudinal in canine material; the dorso-ventral dimensions of the internal structures, expressed as percentages of the proglottid height are for the cuticle 0.34–2.8 (thickest in dogs), sub-cuticle 3.0–11.1, cortex 11.0–23.5, vitellaria 9.4–13.6, muscle zone 9.6–22.0 (thinnest in dogs), medulla 17.6–33.2, testes 14.0–24.6, cirrus sac (percentage of *maximum* proglottid height) 0.50–0.57. The position of the common genital pore, measured from its centre to the anterior proglottid margin, varies between 0.134–0.209 of the proglottid length. The uterine loop counts vary from 3.4–4.9 on either side, with a mean value of 4.3, for the anterior loops, and from 1.1–3.4 with a mean value of 2.0 for the posterior loops, in human material; in canine material the counts are 5.3–8.0 for the anterior loops, 1.3–3.9 for the posterior loops. Circumcloacal papillae absent or faintly visible in human material, prominent in canine material.

On the whole the Manitoba material shows somewhat closer resemblances to Japanese material than to European, but the limited amount of foreign material available for comparison precludes any definite conclusions to this effect.

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References

1. FAUST, E. C. CAMPBELL, H. E. and KELLOG, C. Morphological and biological studies on the species of *Diphyllobothrium* in China. Am. J. Hyg. 9 : 560-583. 1929.
2. JOYEUX, C., RONDEAU DE NOYER, and BAER, J. G. Les Bothriocéphales. Bull. sci. pharmacol. 38 : 175-235. 1931.
3. MAGATH, T. B. Experimental studies on *Diphyllobothrium latum*. Am. J. Trop. Med. 9 : 17-47. 1929.
4. NICHOLSON, D. Fish tapeworm. Intestinal infection in man; the infestation of fish in Manitoba lakes. Can. Med. Assocn. J. 19 : 25-33. 1928.
5. NICHOLSON, D. Diphyllobothrium infection in *Esox lucius*. Can. J. Research, 6 : 166-170. 1932.
6. SOMMER, F. and LANDOIS, L. Ueber den Bau der geschlechtsreifen Glieder von *Bothriocephalus latus*. Z. wiss. Zool. 22 : 41-90. 1872.
7. STEPHENS, J. W. W. Two new human cestodes and a new linguatulid. Ann. Trop. Med. 1 : 549-556. 1908.
8. TARASSOW, W. Beiträge zum Problem des Kampfes gegen *Diphyllobothrium latum* im Nord-Westgebiet. Arch. Schiffs-Tropen-Hyg. 38 : 477-486. 1934.
9. WARDLE, R. A. Significant features in the plerocercoid environment of *Diphyllobothrium latum*. J. Helminthol. 11 : 25-44. 1933.
10. WARDLE, R. A. The limitations of metromorphic characters in the differentiation of Cestoda. Trans. Roy. Soc. Can. 26 : 193-204. 1932.
11. WARDLE, R. A. The viability of tapeworms in vitro. Physiol. Zool. 7 : 36-61. 1934.
12. WARDLE, R. A. Fish tapeworm. Biol. Board. Can. Bull. 45. 1935.

PLASMA LIPIDS IN ACTIVELY IMMUNIZED RABBITS[1]

By Eldon M. Boyd[2], J. H. Orr[3] and G. B. Reed[4]

Abstract

No statistically significant change was noted in the phospholipid and free cholesterol content of the plasma of 16 rabbits following a period of six weeks of active immunization against *Streptococcus viridans* as compared with 28 non-immunized controls.

Boyd (2) has shown that during fever and infection in man there occurs a decrease in the concentration of plasma lipids, to which reaction he has applied the term, lipopenia of fever. Investigating this reaction, Boyd, Orr and Reed (4) found that normal variations of body temperature were not accompanied by significant alterations in the percentage of plasma phospholipid and free cholesterol in healthy rabbits. From this and other evidence it appeared that variations in plasma lipid values during fever and infection were not due to the changes in body temperature *per se*. In the present study an attempt was made to determine whether the development of antigenic reactions is a causal factor in producing changes in lipid metabolism during fever and infection. Velluz (7) was unable to demonstrate any effect of immunization upon serum cholesterol, and Felton and Kauffmann (5) found no change in the alcohol-soluble material of blood, which may be taken as a rough index of the total lipid. Hyman (7), however, reported slight decreases in both phospholipid and free cholesterol in a brief report of work in which apparently little attention was given to variations which might have occurred in control animals.

The procedure in the present investigation was as follows. Sixteen healthy rabbits were actively immunized against a strain of *Streptococcus viridans* which had been isolated from a human case of endocarditis. The animals were injected on three successive days with increasing standardized doses of a vaccine prepared from the heat-killed organisms. This procedure was repeated 10 days later with larger doses and a third series of living organisms was given after a second 10-day interval. This procedure effectively produced active immunity. The agglutination titre of serum rose from an average of less than 1 in 10 before to 1 in 3200 after immunization, and the opsonic index rose from 1 to 3.2. None of the animals died or became seriously sick when the living organisms were injected. The organism was known to be actively pathogenic to rabbits because when injected into rabbits not previously immunized it invariably produced a marked infection and a high mortality rate.

Twenty-eight other rabbits were used as non-immunized controls. There occurred no significant change in the serum content of agglutinins or opsonins in the control group. In neither the control nor immunized group did any appreciable alteration in the rectal temperature occur.

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Blood for lipid analysis was taken after starving the animals for 15 hours, one specimen being obtained before and one after immunization, and at corresponding periods in the control animals. The samples of blood were heparinized and alcohol-ether extracts were prepared by the method of cold dilution (3). The phospholipid and free cholesterol content of these extracts were determined by the oxidative micromethods of Bloor as modified by Boyd (1).

The results obtained have been statistically summarized in Table I. Values reported include the average value before immunization, the average after, the average difference, and the standard deviation of the average difference

TABLE I

EFFECT OF ACTIVE IMMUNIZATION UPON THE PHOSPHOLIPID AND FREE CHOLESTEROL CONTENT OF RABBIT PLASMA

(The results are expressed in mg. per 100 cc. of plasma.)

Group	No. of animals	Average phospholipid				Average free cholesterol			
		Before	After	Difference	Standard deviation of difference	Before	After	Difference	Standard deviation of difference
Immunized	16	71	54	−17	22	16	14	−2	12
Non-immunized	28	70	75	+ 5	23	19	19	0	7

calculated by a formula previously given (1). Presented in this manner, a difference to be significant should be at least twice its standard deviation. It may be seen from the table that the difference, neither with phospholipid nor with free cholesterol, was sufficiently great to satisfy the requirements of this criterion of significance. There was practically no change in the free cholesterol content before and after immunization, and the slight decrease in the means for phospholipid was not great enough to be statistically significant. Hence it may be concluded that following a period of six weeks of active immunization of rabbits with *Streptococcus viridans* there is no significant change in the serum content of phospholipid and free cholesterol. Since variations in the concentration of one lipid in plasma seldom occur without corresponding changes in the concentration of other lipids, it is probable that the immunization as performed would not have produced any changes in the concentration of any lipid in the plasma of rabbits. Keeping in mind that the experiments were performed only on one species and with one organism, they suggest that the changes in the concentration of plasma lipids during fever and infection are not due to the processes concerned with the production of active immunity.

The abstract given at the beginning of this paper may be taken as a summary of the results obtained.

References

1. BOYD, E. M. A differential lipid analysis of blood plasma in normal young women by micro-oxidative methods. J. Biol. Chem. 101 : 323-336. 1933.
2. BOYD, E. M. The lipopenia of fever. Can. Med. Assoc. J. 32 : 500-506. 1935.
3. BOYD, E. M. The extraction of blood lipids. J. Biol. Chem. 114 : 223-234. 1936.
4. BOYD, E. M., ORR, J. H., and REED, G. B. Body temperature and plasma lipids in rabbits. Proc. Soc. Exptl. Biol. Med. 35 : 479-482. 1937.
5. FELTON, L. D., and KAUFFMANN, G. Lipoidal content of antipneumococcic horse serum. J. Immunol. 24 : 543-548. 1933.
6. HYMAN, L. W. Lipids in the plasma of immunized and non-immunized horses. N.Y. State Dept. Health, Ann. Rept. Div. Lab. Research, 23-24. 1935.
7. VELLUZ, L. Sur la détermination simple du rapport esters du cholestérol/cholestérol total dans le sérum sanguin. Compt. rend soc. biol. 112 : 255-256. 1933.